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Dated 11 September 2001

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0030647.2

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Request for grant of a Patent

0030647.2

Form 1/77

Patents Act 1977

1 Title of invention

1 Please give the title of the invention: TREATMENT OF MALE SEXUAL DYSFUNCTION

2 Applicant's details

First or only applicant

2a If you are applying as a corporate body please give:

Corporate name
PFIZER LIMITED

Country *(and State of incorporation, if appropriate)*

UNITED KINGDOM

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases,** please give the following details:

Address
RAMSGATE ROAD
SANDWICH
KENT

UK postcode CT13 9NJ
(if applicable)

Country UNITED KINGDOM

ADP number *(if known)* 5060102000l

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2d, 2e and 2f:
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Second applicant (if any)

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DR. FIONA A. EDWARDS

Agent's address
PFIZER LIMITED
RAMSGATE ROAD
SANDWICH
KENT

Postcode CT13 9NJ

Agent's ADP number 07951460001

3b:
If you have appointed an agent, all correspondence concerning your application will be sent to the agent's United Kingdom address.

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4 Reference number

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5 Claiming an earlier application date

5 Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

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- [ ] number of earlier application or patent number
- [ ] filing date *(day month year)*
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6

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7

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8

Please supply duplicates of claim(s), abstract, description and drawing(s).

8 Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application.

Continuation sheets for this Patents Form 1/77

Claim(s)	12	Description	192
Abstract	1	Drawing(s)	/

8b Which of the following documents also accompanies the application?

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Priority documents *(please state how many)*	/
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TREATMENT OF MALE SEXUAL DYSFUNCTION

FIELD OF INVENTION

The present invention relates to compounds and pharmaceutical compositions for use in the treatment of male sexual dysfunction, in particular male erectile dysfunction (MED).

The present invention also relates to a method of treatment of MED.

The present invention also relates to assays to screen for the compounds of the present invention and which form part of the pharmaceutical compositions of the present invention and which are useful in the treatment of male sexual dysfunction, in particular MED.

For convenience, a list of abbreviations that are used in the following text is presented before the Claims section.

SEXUAL DYSFUNCTION

Sexual dysfunction (SD) is a significant clinical problem which can affect both males and females. The causes of SD may be both organic as well as psychological. Organic aspects of SD are typically caused by underlying vascular diseases, such as those associated with hypertension or diabetes mellitus, by prescription medication and/or by psychiatric disease such as depression. Physiological factors include fear, performance anxiety and interpersonal conflict. SD impairs sexual performance, diminishes self-esteem and disrupts personal relationships thereby inducing personal distress. In the clinic, SD disorders have been divided into female sexual dysfunction (FSD) disorders and male sexual dysfunction (MSD) disorders (Melman *et al* 1999). FSD is best defined as the difficulty or inability of a woman to find satisfaction in sexual expression. Male sexual dysfunction (MSD) is generally associated with erectile dysfunction, also known as male erectile dysfunction (MED) (Benet *et al* 1994).

MALE ERECTILE DYSFUNCTION (MED)

It is known that some individuals can suffer from male erectile dysfunction (MED).

Male erectile dysfunction (MED) is defined as:

"the inability to achieve and/or maintain a penile erection for satisfactory sexual performance" (NIH Consensus Development Panel on Impotence, 1993)"

It has been estimated that the prevalence of erectile dysfunction (ED) of all degrees (minimal, moderate and complete impotence) is 52% in men 40 to 70 years old, with higher rates in those older than 70 (Melman *et al* 1999). The condition has a significant negative impact on the quality of life of the patient and their partner, often resulting in increased anxiety and tension which leads to depression and low self esteem. Whereas two decades ago, MED was primarily considered to be a psychological disorder (Benet *et al* 1994), it is now known that for the majority of patients there is an underlying organic cause. As a result, much progress has been made in identifying the mechanism of normal penile erection and the pathophysiology of MED.

Penile erection is a haemodynamic event which is dependent upon the balance of contraction and relaxation of the corpus cavernosal smooth muscle and vasculature of the penis (Lerner *et al* 1993). Corpus cavernosal smooth muscle is also referred to herein as corporal smooth muscle or in the plural sense corpus cavernosa. Relaxation of the corpus cavernosal smooth muscle leads to an increased blood flow into the trabecular spaces of the corpus cavernosa, causing them to expand against the surrounding tunica and compress the draining veins. This produces a vast elevation in blood pressure which results in an erection (Naylor, 1998).

The changes that occur during the erectile process are complex and require a high degree of co-ordinated control involving the peripheral and central nervous systems, and the endocrine system (Naylor, 1998). Corporal smooth muscle contraction is modulated by sympathetic noradrenergic innervation via activation of postsynaptic α_1 adrenoceptors. MED may be associated with an increase in the endogenous smooth muscle tone of the corpus cavernosum. However, the process of corporal smooth muscle relaxation is mediated primarily by non-adrenergic, non-cholinergic (NANC) neurotransmission. There are a number of other NANC neurotransmitters found in

the penis, other than NO, such as calcitonin gene related peptide (CGRP) and vasoactive intestinal peptide (VIP). The main relaxing factor responsible for mediating this relaxation is nitric oxide (NO), which is synthesised from L-arginine by nitric oxide synthase (NOS) (Taub *et al* 1993; Chuang *et al* 1998). It is thought that reducing corporal smooth muscle tone may aid NO to induce relaxation of the corpus cavernosum. During sexual arousal in the male, NO is released from neurones and the endothelium and binds to and activates soluble guanylate cyclase (sGC) located in the smooth muscle cells and endothelium, leading to an elevation in intracellular cyclic guanosine 3',5'-monophosphate (cGMP) levels. This rise in cGMP leads to a relaxation of the corpus cavernosum due to a reduction in the intracellular calcium concentration ($[Ca^{2+}]_i$), via unknown mechanisms thought to involve protein kinase G activation (possibly due to activation of Ca^{2+} pumps and Ca^{2+}-activated K^+ channels; Chuang *et al.*, 1998).

MED mainly arises from an inability of NO released during sexual arousal to effectively relax corpus cavernosal smooth muscle. It has therefore been proposed that MED may be treatable by potentiating or facilitating nitrergic signalling thereby leading to an elevation in intracellular cGMP levels. Nitrergic signalling as defined herein means the cellular mechanisms that are activated by the NO released or generated during sexual arousal / stimulation and in particular relates to the activation of the guanalyl cyclase / cGMP pathway. In this respect, sildenafil citrate (also known as Viagra™) has recently been developed by Pfizer as the first oral drug treatment for MED. Sildenafil acts by inhibiting cGMP breakdown in the corpus cavernosa by selectively inhibiting phosphodiesterase 5 (PDE5), thereby limiting the hydrolysis of cGMP to 5'GMP (Boolel *et al.*, 1996; Jeremy *et al.*, 1997) and thereby increasing the intracellular concentrations of cGMP and facilitating corpus cavernosal smooth muscle relaxation.

Currently, all other available MED therapies on the market, such as treatment with prostaglandin based compounds i.e. alprostadil which can be administered intra-urethrally (available from Vivus Inc., as Muse™) or via small needle injection (available from Pharamcia & Upjohn, as Caverject™), are either inconvenient and/or invasive. Other treatments include vacuum constriction devices, vasoactive drug injection or penile prostheses implantation (Montague et al., 1996). Although injectable vasoactive drugs show high efficacy, side effects such as penile pain, fibrosis and priapism are common, and injection therapy is not as convenient as oral

therapy therefore sildenafil currently represents the most preferred therapy on the market.

The present invention relates to compounds and pharmaceutical compositions useful for the treatment of MED which act via a complementary pathway to the inhibition of cGMP breakdown by PDE enzyme hydrolysis. A complementary pathway(s) as defined herein means a pathway which is implicated in the endogenous erectile process and is stimulated by sexual arousal. Further, a complementary pathway as defined herein is substantially, and preferably wholly, independent of the release of NO from nitrergic nerves by sexual stimulation. Moreover the complementary pathway as defined herein exists in complement with the nitrergic/NO pathway which is also stimulated by sexual arousal. In other words the complementary pathway means a pathway working via a separate mechanism to the PDE5 enzyme-mediated hydrolysis of cGMP (as discussed hereinbefore in relation to sildenafil) wherein said complementary pathway essentially acts via a non-soluble guanylate cylase (nsGC) activated pathway. Acting essentially via a non-soluble nsGC activated pathway means that the majority of action is via non-soluble nsGC whilst a minority may act via soluble guanylate cyclase (sGC). The terms majority and minority are relative terms wherein a majority is preferably greater than about 80%, more preferably greater than about 90% and especially greater than about 95%.

It has previously been proposed that mechanisms other than inhibition of cGMP breakdown, such as, mechanisms involving neuropeptides (e.g. endothelin-1, vasoactive intestinal peptide (VIP); (Christ *et al.*, 1995), gap junctions (Christ *et al.*, 1991) and ion channels (Christ *et al.*, 1993; Chiou *et al.*, 1998) may also modulate corporal smooth muscle tone.

VIP is localised to the neurones innervating the corpus cavernosa and penile vasculature (Argiolas *et al.*, 1995). However the role of VIP in the erectile mechanism is less clear, compared to that for NO. Whilst it has been proposed that VIP may have a role in the erectile process clinical data to date has been negative. Exogenously applied VIP potently relaxes isolated human corpus cavernosum muscle strips (Willis *et al.*, 1983; Steers *et al.*, 1984, Hedlund *et al.*, 1985, Adaikan *et al.*, 1986). Despite these results in isolated tissues, repeated clinical studies have shown that intracavernosal injection of VIP does not produce erections in normal or impotent men (Wagner *et al.*, 1987; Kiely *et al.*, 1989; Roy *et al.*, 1990). Furthermore, there is evidence against the involvement of VIP as the key NANC

transmitter of erection (reviewed in Naylor, 1998). Whilst the art teaches that administration of VIP alone, with, or without sexual stimulation, to a male suffering from MED does not produce erection sufficient for intercourse, it has been reported that combinations of VIP with phentolamine or papaverine can induce erections sufficient for intercourse, in the absence of sexual stimulation, when administered intracavernosally, (Kiely *et al.*, 1989). Phentolamine is an α–andrenergic antagonist and a known treatment for MED (available as Vasomax™ from Zonagen Inc.). It has been proposed that the erections observed for VIP/phentolamine or papaverine combinations were due to increases in venous outflow resistance attributable to α–andrenergic antagonists i.e. phentolamine or paparverine rather than a VIP-induced effect.

The present invention provides compounds and pharmaceutical compositions useful for the treatment of MED which act via a complementary pathway to the inhibition of cGMP breakdown by PDE enzyme hydrolysis.

Surprisingly the applicants have also found that inhibition of NEP EC3.4.24.11 with a selective neural endopeptidase inhibitor, hereinafter referred to as an NEPi, significantly enhances the nerve-stimulated erectile process.

According to the present invention there is provided the use of a selective inhibitor of the neural endopeptidase EC3.4.24.11, hereinafter referred to as a NEPi, for the treatment of MED.

There is further provided the use of a NEPi in the manufacture of a medicament for the treatment of MED.

There is no documented evidence for the expression or a functional role of NEP EC3.4.24.11 in the penis or corpus cavernosum or in the erectile mechanism/process.

There is no documented evidence for a functional or biochemical effect for selective NEP inhibitors on the penis or corpus cavernosum or alternatively in the erectile mechanism/process.

Summary Aspects of The Present Invention

A seminal finding of the present invention is the ability to treat an male suffering from sexual dysfunction, in particular MED, with use of an NEPi.

The present invention provides compounds and pharmaceutical compositions useful for the treatment of MED which act via a complementary pathway to the inhibition of the cGMP breakdown by PDE enzyme hydrolysis.

In particular the present invention provides NEPi compounds for use in the treatment of MED.

The present invention is advantageous as it provides a means for restoring a normal sexual arousal response - namely increased penile blood flow leading to erection of the penis. Hence, the present invention provides a means to restore, or potentiate, the normal sexual arousal response.

Without wishing to be bound to any particular theory it is believed that use of the compounds of the present invention act via inhibiting the mechanism that terminates the biological activity of a number of bioactive peptides and in particular vasoactive peptides, more particularly neuropeptides, that are released during sexual arousal in order to treat MED wherein said mechanism is a complementary pathway to the NO pathway as defined hereinbefore. Vasoactive peptides include both vasorelaxant and vasoconstrictor peptides, preferred.

It is further proposed that the use of the compounds according to the present invention acts via enhancing a non-NO dependant NANC pathway to treat MED.

It is proposed herein that the use of NEPi compounds for the treatment of MED according to the present invention acts upon a complementary pathway, as defined hereinbefore, or acts to potentiate or facilitate upon said complementary pathway. By this reasoning it if further proposed that the use of NEP inhibitor compounds for the treatment of MED may be advantageous via their potential to overcome the deficiency/reduction in nitrergic signalling by acting upon said complementary pathway.

Surprisingly the applicants have also found that inhibition of NEP EC3.4.24.11 with a selective neural endopeptidase inhibitor, hereinafter referred to as an NEPi, significantly potentiates PDE5 inhibitor-mediated enhancement of the erectile process.

It is therefore an object of the present invention to provide NEPi compounds and pharmaceutical compositions comprising NEPi compounds that inhibit NEP EC3.4.24.11 for use in the treatment of MED. For examples and discussion thereof see Test Results Section, Examples 1, 2 and 3.

Thus according to a further embodiment the present invention provides the use of one or more NEPi's and one or more PDE5i's for the treatment of MED.

Preferably said combined treatment comprises a combination of one or more NEPi's with one or more PDE5i's. More preferably such combination provides for the concomitant administration of one or more NEPi's with one or more PDE5I's for the treatment of MED.

Highly preferred herein is the use of a pharmaceutical composition comprising one or more NEPi's with one or more PDE5i's for the treatment of MED.

Especially preferred for use in the pharmaceutical compositions for the treatment of MED according to the present invention is the combination of a potent and selective NEPi with a potent and selective PDE5i.

In a preferred embodiment herein said combined administration of NEPi and PDE5i is concomitant. Concomitant administration as defined herein encompasses simultaneous (separate) administration, simultaneous combined administration, separate administration, combined administration, sequential administration and co-formulated combined administration of a cGMP PDE5i and a NEPi.

As detailed hereinbefore the present invention further proposes that, concomitant administration of a PDE5i and NEPi can effect an increase in the efficacy vs that obtainable by PDE5-alone associated MED therapy. For example and discussion thereof see Test Results Section, Examples 4 and 5.

Thus, it is a further object of the present invention to provide pharmaceutical compositions comprising an NEPi and a PDE5i for use in the treatment of MED.

According to a further aspect of the present invention it is proposed herein that, concomitant application of an NEPi and a PDE5i can provide faster onset of action that that achievable via the PDE5i alone. In other words the present invention additionally provides the use of a fast-acting composition for the treatment of MED. A fast acting MED composition as defined herein, and as exemplified hereinafter, means that following i.v. administration of the composition (NEPi and PDE5i) the time to maximal effect on intracavernosal pressure is reduced versus the equivalent time obtained for the same dose of the PDE5i alone. For example and discussion thereof see Test Results Section, Examples 5

Thus, it is a yet further object of the present invention to provide fast acting pharmaceutical compositions comprising an NEPi and a PDE5i for use in the treatment of MED.

It is further proposed herein that use of a NEPi/PDE5i combination may enhance the efficacy of the PDE5i thereby enabling a reduction in the dose of PDE5 inhibitor required for a specific efficacy. A formulation comprising a NEPi and a reduced amount of a PDE5i as defined herein means that a reduced amount of a given PDE5i is required to effect a particular response when combined with an effective amount of a NEPi according to the present invention than the required amount of PDE5i alone. Such reduced dose compositions for the treatment of MED may be desirable for particular patient groups such as for example men with mild MED.

Thus, it is a still further object of the present invention to provide a pharmaceutical composition comprising an NEPi and a reduced dose of PDE5i for use in the treatment of MED.

<u>NEP EC3.4.24.11</u>

NEP EC3.4.24.11, also known as enkephalinase or neprilysin, is a zinc-dependent neutral endopeptidase. This enzyme is involved in the breakdown of several bioactive oligopeptides, cleaving peptide bonds on the amino side of hydrophobic amino acid residues (Reviewed in Turner et al., 1997). The key neuronally released bioactive agents or neuropeptides metabolised by NEP include natriuretic peptides

such as atrial natriuretic peptides (ANP) as well as brain natriuretic peptide and C-type natriuretic peptide, bombesin, bradykinin, calcitonin gene-related peptide, endothelins, enkephalins, neurotensin, substance P and vasoactive intestinal peptide. Some of these peptides have potent vasodilatory and neurohormone functions, diuretic and natriuretic activity or mediate behaviour effects.

Without being limited to any particular theory it is proposed herein that by inhibiting NEP EC3.4.24.11 other neuronally released vasoactive agents that are released during sexual arousal are enhanced, most likely VIP. It is further proposed herein that the complementary effects of NEP substrates or substrates of NEP metabolism, most likely VIP or other bioactive peptides, with the NO/cGMP pathway that is responsible for the observed effects described for the use of the compounds and pharmaceutical combinations according to the present invention for the treatment of MED.

The NEPi, and where present PDE5i compounds, useful for the treatment of MED according to the present invention, may also be used in combination with one or more additional pharmaceutically active agents. The additional pharmaceutically active agent(s) as defined hereinbefore, if present, may be referred to as an "additional agent". One or more of such additional agents may be one or more of: PDEi, another NEPi, or an NPYi. Combinations of agents are discussed in more detail below.

General references herein to agents may be applicable to additional agents as well as to NEPi or PDE5i compounds.

In accordance with the use of NEPi compounds for the treatment of MED according to the as discussed hereinbefore, the NEPi acts on a target, preferably specifically on that target. For example where a combination of a NEPi and a PDE5i are present the targets are the NEP and PDE5 enzymes. This target is sometimes referred to as the "target of the present invention". However, the additional agents of the present invention may act on one or more other targets. These other targets may be referred to as an "additional target". Likewise, if an additional agent is used, then that additional agent can target the same target of the present invention and/or an additional target (which need not be the same additional target that is acted on by the agent of the present invention). Targets are described herein. It is to be understood

that general references herein to targets may be applicable to the additional targets as well as to the target of the present invention.

DETAILED ASPECTS OF THE PRESENT INVENTION

In one aspect, the present invention relates to NEPi compounds and pharmaceutical compositions including NEPi compounds and pharmaceutical combinations comprising NEPi and PDE5i for use (or when in use) in the treatment of male sexual dysfunction, in particular MED. In said pharmaceutical compositions the NEPi (and PDE5I, if present, and/or additional agent) is optionally admixed with a pharmaceutically acceptable carrier, diluent or excipient. Here, the composition (like any of the other compositions mentioned herein) may be packaged for subsequent use in the treatment of male sexual dysfunction, in particular MED.

In another aspect, the present invention relates to the use of an agent in the manufacture of a medicament (such as a pharmaceutical composition) for the treatment of male sexual dysfunction, in particular MED.

In a further aspect, the present invention relates to a method of treating a male suffering from male sexual dysfunction, in particular MED; the method comprising delivering to the male an NEPi that is capable of enhancing the endogenous erectile process in the corpus cavernosum; wherein the NEPi is present in an amount to enhance the endogenous erectile process as defined hereinbefore; wherein the NEPi is optionally admixed with a pharmaceutically acceptable carrier, diluent or excipient; and wherein said NEPi is as herein defined.

In a further aspect, the present invention relates to an assay method for identifying an agent (hereinafter referred to as a NEPi) that can be used to treat male sexual dysfunction, in particular MED, the assay method comprising: determining whether a test agent can directly enhance the endogenous erectile process; wherein said enhancement is defined as a potentiation of intracavernosal pressure (ICP) (and/or cavernosal blood flow) in the presence of a test agent as defined herein; such potentiation by a test agent is indicative that the test agent may be useful in the treatment of male sexual dysfunction, in particular MED and wherein said test agent is a NEPi.

By way of example, the present invention relates to an assay method for identifying an agent that can directly enhance the endogenous erectile process in order to treat male sexual dysfunction, in particular MED, the assay method comprising: contacting a test agent with a moeity capable of inhibiting the metabolic breakdown of a peptide (preferably a fluorescent labelled peptide); and measuring the activity and/or levels of peptide remaining after a fixed time (for example via fluorometric analysis); wherein the change in the level of fluorescence is indicative of the potency (IC_{50}) of the test agent and is indicative that the test agent may be useful in the treatment of male sexual dysfunction, in particular MED; and wherein said agent is an NEPi.

In a further aspect, the present invention relates to a process comprising the steps of: (a) performing the assay according to the present invention; (b) identifying one or more agents that can directly enhance the endogenous erectile process; and (c) preparing a quantity of those one or more identified agents; and wherein said agent is an NEPi.

With this aspect, the agent identified in step (b) may be modified so as to, for example, maximise activity and then step (a) may be repeated. These steps may be repeated until the desired activity or pharmacokinetic profile has been achieved.

Thus, in a further aspect, the present invention relates to a process comprising the steps of: (a1) performing the assay according to the present invention; (b1) identifying one or more agents that can directly enhance the endogenous erectile process ; (b2) modifying one or more of said identified agents; (a2) optionally repeating step (a1); and (c) preparing a quantity of those one or more identified agents (i.e. those that have been modified); and wherein said agent is an NEPi.

In a further aspect, the present invention relates to a method of treating male sexual dysfunction, in particular MED, by potentiating the nerve stimulated endogenous erectile process *in vivo* (rabbit and / or dog) by measuring the ICP or cavernosal blood flow with an agent; wherein the agent is capable of directly inhibiting the metabolic breakdown of a fluorescent peptide (as detailed hereinbefore) in an *in vitro* assay method; wherein the *in vitro* assay method is the assay method according to the present invention; and wherein said agent is an NEPi.

In a further aspect, the present invention relates to the use of an agent in the preparation of a pharmaceutical composition for the treatment of male sexual dysfunction, in particular MED, wherein the agent is capable of directly inhibiting the

metabolic breakdown of a fluorescent peptide when assayed *in vitro* by the assay method according to the present invention; and wherein said agent is an NEPi.

In a further aspect, the present invention relates to an animal model used to identify agents capable of treating male sexual dysfunction (in particular MED), said model comprising an anaesthetised male animal including means to measure changes in intracavernosal pressure and/or cavernosal blood flow of said animal following stimulation of the pelvic nerve thereof; and wherein said agent is an NEPi.

In a further aspect, the present invention relates to an assay method for identifying an agent that can directly enhance the endogenous erectile process in order to treat MED, the assay method comprising: administering an agent to the animal model of the present invention; and measuring the change in the endogenous erectile process; wherein said change is defined as a potentiation of intracavernosal pressure (ICP) (and/or cavernosal blood flow) in the animal model in the presence of a test agent as defined; and wherein said agent is an NEPi.

In a further aspect, the present invention relates to a diagnostic method, the method comprising isolating a sample from a male; determining whether the sample contains an entity present in such an amount as to cause male sexual dysfunction, preferably MED; wherein the entity has a direct effect on the endogenous erectile process in the corpus cavernosum of the male; and wherein said entity can be modulated to achieve a beneficial effect by use of an agent; and wherein said agent is an NEPi.

In a further aspect, the present invention relates to a diagnostic composition or kit comprising means for detecting an entity in an isolated male sample; wherein the means can be used to determine whether the sample contains the entity and in such an amount to cause male sexual dysfunction, preferably MED, or is in an amount so as to cause sexual dysfunction, preferably MED; wherein the entity has a direct effect on the endogenous erectile process and wherein said entity can be modulated to achieve a beneficial effect by use of an agent; and wherein said agent is an NEPi.

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

PREFERABLE ASPECTS

Preferably, the agents for use in the treatment of MED according to the present invention are NEP EC3.4.24.11 inhibitors.

In one embodiment, preferably the agent for the use according to the present invention may be used via oral administration.

In another embodiment, the agent for the use according to the present invention may be used via topical or intra-urethral, preferably topical administration.

For some applications, preferably the agent for the use according to the present invention is a selective NEPi.

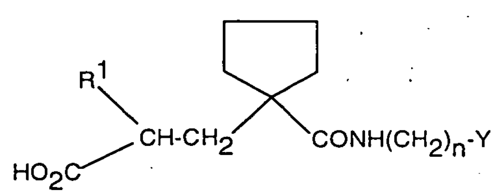
For some applications, preferably the agent for use herein is an NEPi wherein said NEP is EC 3.4.24.11.

For some applications, preferably the agent for use herein is a selective NEPi wherein said NEP is EC 3.4.24.11.

Preferably the agent for use in the treatment of MED according to the present invention is an inhibitor – i.e. it is capable of exhibiting an inhibitory function.

Preferably the agent for use in the treatment of MED according to the present invention is capable of directly enhancing the endogenous erectile process as detailed hereinbefore.

Preferred for use as NEPi's in the treatment of MED according to the present invention are compounds of general formula I:



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6}

hydroxyalkoxy, C_{1-6} alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

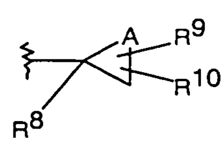
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or $-NR^2R^3$ wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

the $-(CH_2)_n-$ linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

Y is the group

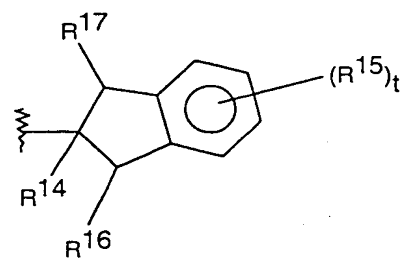


wherein A is $-(CH_2)_q-$ where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, $-CH_2OH$, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each

independently H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl, or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

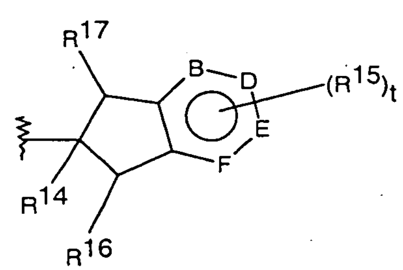
Y is the group, $-C(O)\ NR^{11}\ R^{12}$ wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or

Y is the group,



wherein R^{14} is H, CH_2OH, or $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

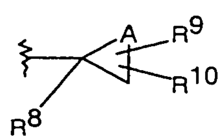
wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl (preferably pyridyl); and

u is 0, 1, 2 or 3.

Preferably R^1 is C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl, C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl or C_{1-6}alkyl substituted with aryl. Particularly preferred R^1 substituents are C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}alkoxy(C_{1-3})alkyl (especially methoxyethyl) or C_{1-6}alkoxyC_{1-6}alkoxyC_{1-3}alkyl (especially methoxyethoxymethyl). It is especially preferred that R^1 is C_{1-4}alkyl (preferably propyl).

When Y is the group



and the carbocyclic ring is fully saturated, then preferably one of R^9 or R^{10} is $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl. More, preferably the carbocyclic ring is 5, 6 or 7 membered wherein one of R^9 or R^{10}, $-C(O)NR^{11}R^{12}$, with the other being C_{1-6}alkyl, phenyl optionally substituted by

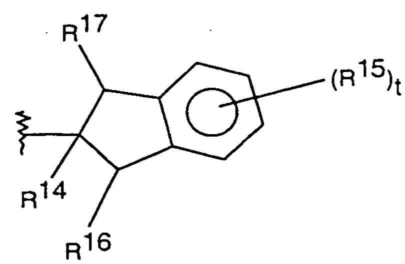
C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl. More preferably, R^9 and R^{10} are attached to adjacent carbon atoms in the ring. More preferably, R^8 is CH_2OH.

When Y is the group $-NR^{18}S(O)_uR^{19}$, preferably R^{18} is H. More preferably, R^{19} is benzyl or phenyl. More preferably u is 2.

Preferably Y is the optionally substituted 5-7 membered heterocyclic ring. More preferably the ring is an optionally substituted aromatic ring, particularly pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazolyl, thiazolyl, thiaiazolyl, oxazolyl, isoxazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, pyridonyl, quinoxalinyl or quinazolinyl [especially oxadiazole (preferably 1,2,5- or 1,3,4-oxadiazole), pyridone (preferably 2-pyridone) or thiadiazole (preferably 1,3,4-thiadiazole) each of which may be substituted as defined in the first aspect. Preferably the heterocyclic ring is substituted by one or more C_{1-6}alkyl, phenyl or phenylC_{1-4}alkyl, more preferably by C_{1-4}alkyl or benzyl. Preferably Y is an *N*-substituted pyridone, preferably by benzyl or C_{1-4}alkyl.

Preferably Y is a lactam linked at the nitrogen.

Preferably Y is



wherein R^{14} is preferably CH_2OH or $C(O)NR^{11}R^{12}$, especially $C(O)NR^{11}R^{12}$. Preferably R^{16} and R^{17} are hydrogen. Preferably t is 0.

The chiral carbon attached to R^1 is preferably the R-enantiomer.

Particularly preferred NEPi compounds for use in the treatment of MED according to the present invention (referred to hereinafter as the list of 10 preferred NEPi compounds) are:

2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-4-methoxybutanoic acid (NEPi Example 35),

2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoic acid (NEPi Example 40),

(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (NEPi Example 44),

2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoic acid (NEPi Example 43),

cis-3-(2-methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}cyclohexyl)-amino]carbonyl}cyclopentyl)methyl]propanoic acid (NEPi Example 38),

(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid (NEPi Example 31),

(+)-2-[(1-{[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid (NEPi Example 30),

2-({1-[(3-benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (Example 21),

2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-pentanoic acid (NEPi Example 22), and

2-{[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (NEPi Example 9).

In the above definition, unless otherwise indicated, alkyl groups having three or more carbon atoms may be straight or branched-chain. The term aryl as used herein means an aromatic hydrocarbon group such as phenyl or naphthyl which may optionally be substituted with, for example, one or more of OH, CN, CF_3, C_1-C_4 alkyl, C_1-C_4 alkoxy, halo, carbamoyl, aminosulphonyl, amino, mono or di(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Halo means fluoro, chloro, bromo or iodo.

In the above definition, unless otherwise indicated the term heterocyclyl means a 5 or 6 membered nitrogen, oxygen or sulphur containing heterocyclic group which, unless otherwise stated, may be saturated, unsaturated or aromatic and which may optionally include a further oxygen or one to three nitrogen atoms in the ring and which may optionally be benzofused or substituted with for example, one or more

halo, C_1-C_4 alkyl, hydroxy, carbamoyl, benzyl, oxo, amino or mono or di-(C_1-C_4 alkyl)amino or (C_1-C_4 alkanoyl)amino groups. Particular examples of heterocycles include pyridyl, pyridonyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, furanyl, tetrahydrofuranyl, tetrahydropyranyl, dioxanyl, thienyl, oxazolyl, isoxazolyl, thiazolyl, oxadiazolyl, thiadiazolyl, indolyl, isoindolinyl, quinolyl, isoquinolyl, quinoxalinyl, quinazolinyl and benzimidazolyl, each being optionally substituted as previously defined.

Details on a suitable assay system for identifying and/or studying an I:NEP are presented in the hereinafter in the section entitled NEP Assay.

Further examples of NEP inhibitors are disclosed and discussed in the following review articles:

Pathol. Biol., 46(3), 1998, 191.
Current Pharm. Design, 2(5), 1996, 443.
Biochem. Soc. Trans., 21(3), 1993, 678.
Handbook Exp. Pharmacol., 104/1, 1993, 547.
TiPS, 11, 1990, 245.
Pharmacol. Rev., 45(1), 1993, 87.
Curr. Opin. Inves. Drugs, 2(11), 1993, 1175.
Antihypertens. Drugs, (1997), 113.
Chemtracts, (1997), 10(11), 804.
Zinc Metalloproteases Health Dis. (1996), 105.
Cardiovasc. Drug Rev., (1996), 14(2), 166.
Gen. Pharmacol., (1996), 27(4), 581.
Cardiovasc. Drug Rev., (1994), 12(4), 271.
Clin. Exp. Pharmacol. Physiol., (1995), 22(1), 63.
Cardiovasc. Drug Rev., (1991), 9(3), 285.
Exp. Opin. Ther. Patents (1996), 6(11), 1147.

Yet, further examples of NEPi's are disclosed in the following documents:

EP-509442A

US-192435

US-4929641

EP-599444B

US-884664

EP-544620A

US-798684

J. Med. Chem. 1993, 3821.

Circulation 1993, 88(4), 1.

EP-136883

JP-85136554

US-4722810

Curr. Pharm. Design, 1996, 2, 443.

EP-640594

J. Med. Chem. 1993, 36(1), 87.

EP-738711-A

JP-270957

CAS # 115406-23-0

DE-19510566

DE-19638020

EP-830863

JP-98101565

EP-733642

WO9614293

JP-08245609

JP-96245609

WO9415908

JP05092948

WO-9309101

WO-9109840

EP-519738

EP-690070

J. Med. Chem. (1993), 36, 2420.

JP-95157459

Bioorg. Med. Chem. Letts., 1996, 6(1), 65.

Further I:NEPs are disclosed in the following documents:

EP-A-0274234

JP-88165353

Biochem.Biophys.Res. Comm.,1989, 164, 58

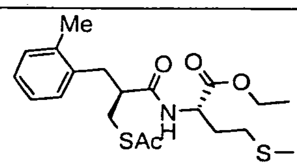
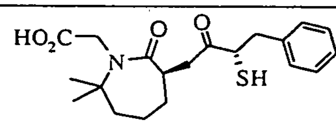
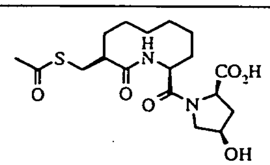
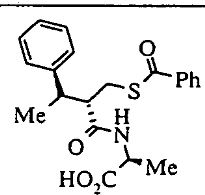
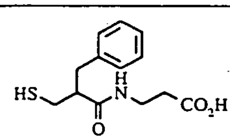
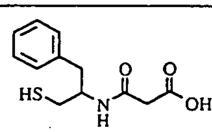
EP-629627-A

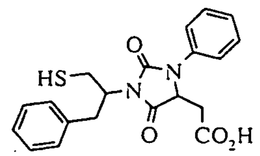
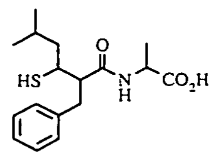
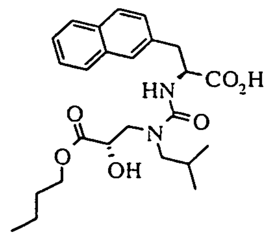
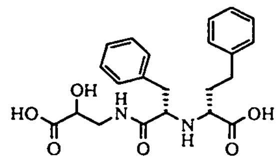
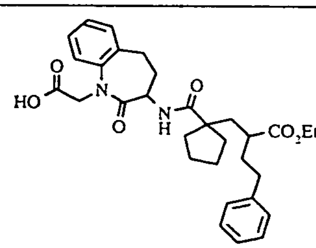
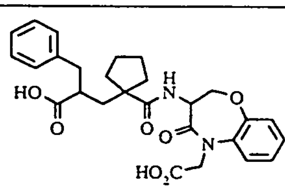
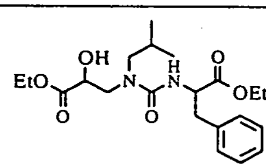
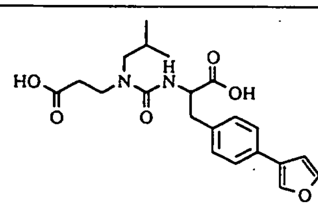
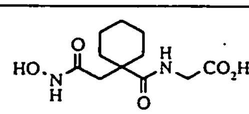
US-77978

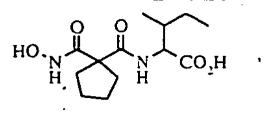
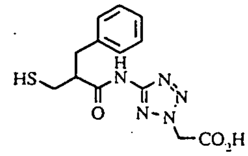
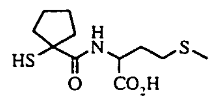
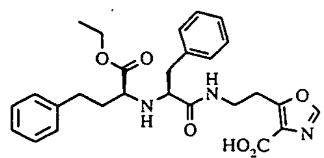
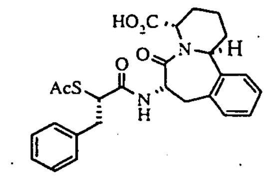
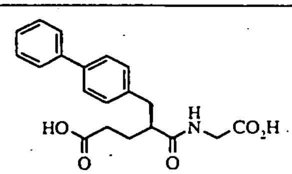
Perspect. Med. Chem. (1993), 45.

EP-358398-B

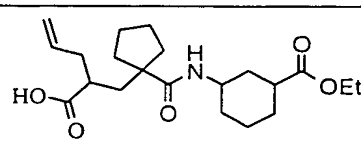
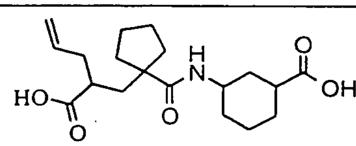
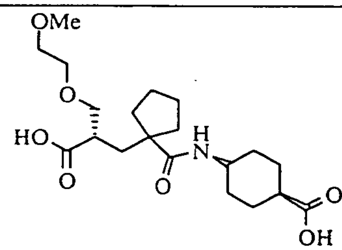
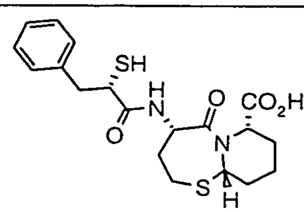
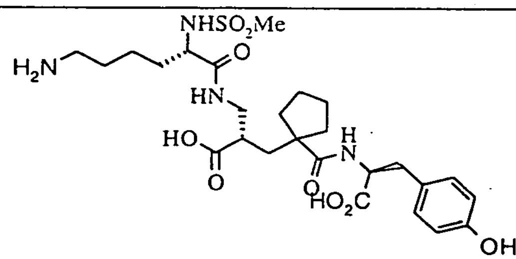
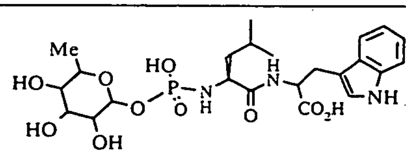
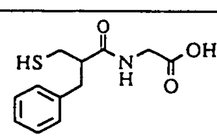
Further examples of I:NEPs are selected from the following structures:

Compound	Structure	Mode of Action References
FXII		I:NEP EP-509442A US-192435 US-4929641
FXIII		I:NEP (also an ACE inhibitor) EP-599444B US-884664
FXIV		I:NEP EP-544620A US-798684 J. Med. Chem. 1993, 3821.
FXV		I:NEP (also an ACE inhibitor) Mixanpril Circulation 1993, 88(4), 1.
FXVI		I:NEP EP-136883 JP-85136554 US-4722810
FXVII		I:NEP Retrothiorphan Curr. Pharm. Design, 1996, 2, 443.

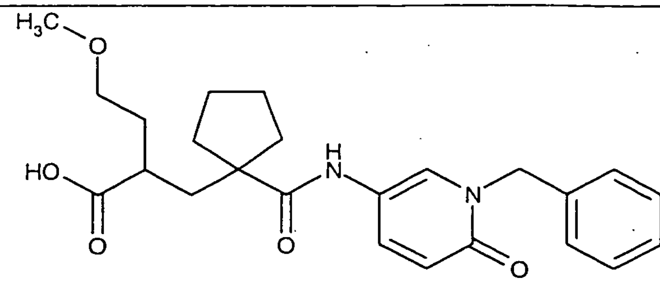
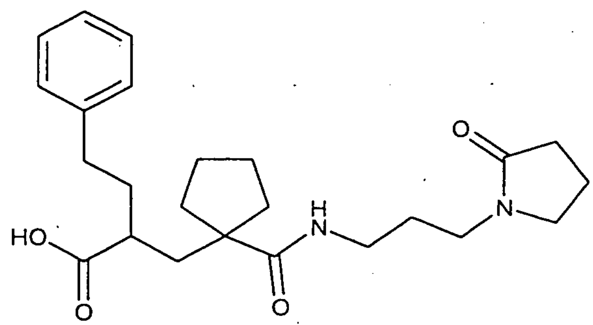
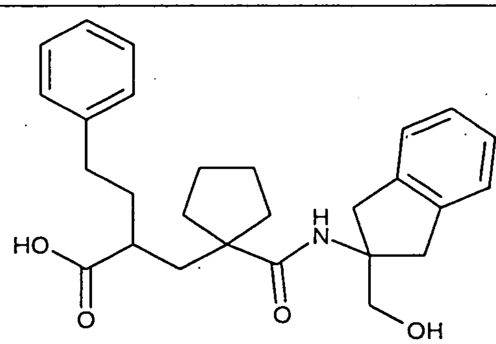
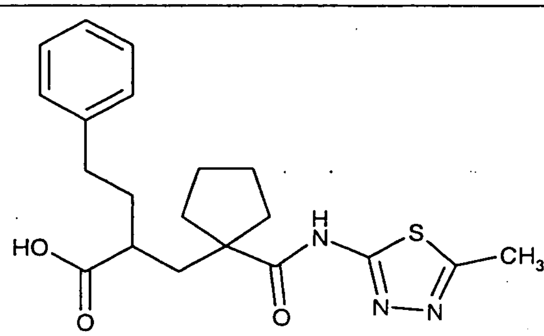
FXVIII		I:NEP (also an ACE inhibitor) EP-640594
FXIX		I:NEP J. Med. Chem. 1993, 36(1), 87.
FXX		I:NEP (also an ACE inhibitor) EP-738711-A JP-270957
FXXI		I:NEP CAS # 115406-23-0
FXXII		I:NEP (also an ECE inhibitor) DE-19510566 DE-19638020 EP-830863 JP-98101565
FXXIII		I:NEP (also an ECE inhibitor) EP-733642
FXXIV		I:NEP WO96/14293
FXXV		I:NEP JP-08245609 JP-96245609
FXXVI		I:NEP WO9415908

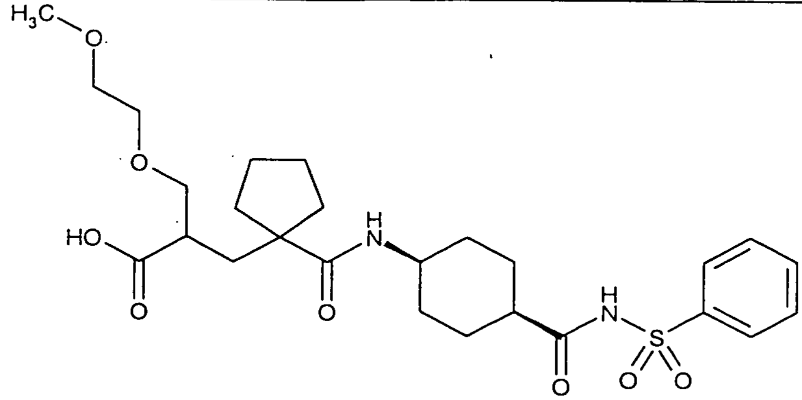
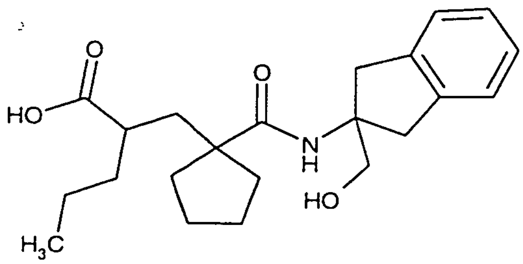
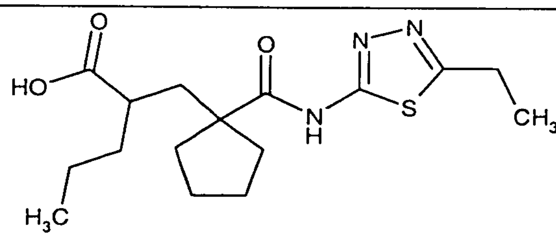
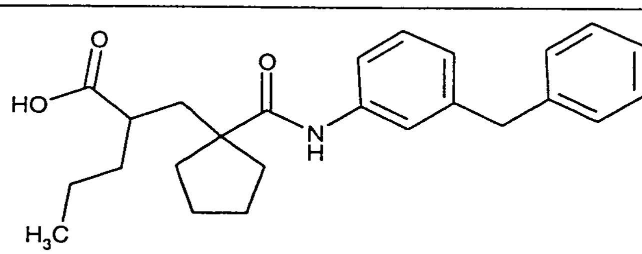
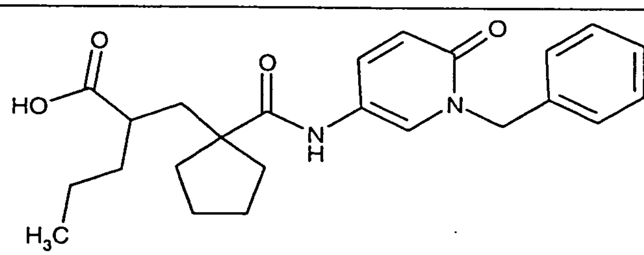
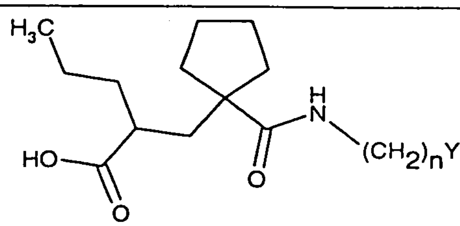
FXXVII		I:NEP JP05092948
FXXVIII		I:NEP WO-9309101
FXXIX		I:NEP WO-9109840
FXXXI		I:NEP EP-519738 EP-690070
FXXXII		I:NEP (also an ACE inhibitor) J. Med. Chem. (1993), 36, 2420.
FXXXIII		I:NEP JP-95157459 Bioorg. Med. Chem. Letts., 1996, 6(1), 65.

Preferred additional I:NEPs are selected from the following structures:

Compound	Structure	Mode of Action References
FV		I:NEP EP-A-0274234 (Example 300)
FVI		I:NEP EP-A-0274234 (Example 379)
FVII		I:NEP Candoxatrilat EP-274234 JP-88165353 Biochem.Biophys.Res. Comm.,1989, 164, 58
FVIII		I:NEP Omapatrilat (also an inhibitor of ACE) EP-0629627-A US-77978
FIX		I:NEP Sampatrilat (also an inhibitor of ACE) Perspect. Med. Chem. (1993), 45. EP-0358398-B
FX		I:NEP Phosphoramidon (which is commercially available)
FXI		I:NEP Thiorphan (which is commercially available)

More preferred additional I:NEPs are selected from the following structures:

COMPOUND	STRUCTURE
F57	
F58	
F59	
F60	

F61	
F62	
F63	
F64	
F65	
F66	 (Ic)

These compounds were prepared according to the teachings presented in the Experimental section (*infra*). These compounds were tested as agents and were found to be useful for enhancing the endogenous erectile process, and thereby being useful in the treatment of MED. Some of the experimental data concerning these compounds are presented in the Experimental section (*infra*).

Preferably, the NEP inhibitors for use in the treatment of MED according to the present invention have an IC_{50} at less than 100 nanomolar, more preferably, at less than 50 nanomolar, more preferably still less than 25 nanomolar and especially preferred less than or equal to 10 nanomolar.

Preferably the NEPi compounds for the use according to the present invention have at least about a 100 fold selectivity to the desired target, preferably at least about a 150 fold selectivity to the desired target, preferably at least about a 200 fold selectivity to the desired target, preferably at least about a 250 fold selectivity to the desired target, preferably at least about a 300 fold selectivity to the desired target, preferably at least about a 350 fold selectivity to the desired target.

More preferably the NEPi compounds for the use according to the present invention (and optionally the optional additional agent) has at least about a 400 fold selectivity to the desired target, preferably at least about a 500 fold selectivity to the desired target, preferably at least about a 600 fold selectivity to the desired target, preferably at least about a 700 fold selectivity to the desired target, preferably at least about a 800 fold selectivity to the desired target, preferably at least about a 900 fold selectivity to the desired target, preferably at least about a 1000 fold selectivity to the desired target.

Preferably, the NEP inhibitors for use in the treatment of MED according to the present invention have greater than 100-fold, more preferably greater than 300-fold and more preferably still greater than 3000-fold selectivity for NEP over either endothelin converting enzyme (ECE) or angiotensin converting enzyme (ACE).

More preferably still the NEP inhibitors for use in the treatment of MED according to the present invention have greater than 100-fold, more preferably greater than 300-fold and more preferably still greater than 3000-fold selectivity for NEP over both endothelin converting enzyme (ECE) and angiotensin converting enzyme (ACE).

Background teachings on NEP have been presented by Victor A. McKusick et al on http://www3.ncbi.nlm.nih.gov/Omim/searchomim.htm. The following information concerning NEP has been extracted from that source.

"Common acute lymphocytic leukemia antigen is an important cell surface marker in the diagnosis of human acute lymphocytic leukemia (ALL). It is present on leukemic cells of pre-B phenotype, which represent 85% of cases of ALL. CALLA is not restricted to leukemic cells, however, and is found on a variety of normal tissues. CALLA is a glycoprotein that is particularly abundant in kidney, where it is present on the brush border of proximal tubules and on glomerular epithelium. Letarte et al. (1988) cloned a cDNA coding for CALLA and showed that the amino acid sequence deduced from the cDNA sequence is identical to that of human membrane-associated neutral endopeptidase (NEP; EC 3.4.24.11), also known as enkephalinase. NEP cleaves peptides at the amino side of hydrophobic residues and inactivates several peptide hormones including glucagon, enkephalins, substance P, neurotensin, oxytocin, and bradykinin. By cDNA transfection analysis, Shipp et al. (1989) confirmed that CALLA is a functional neutral endopeptidase of the type that has previously been called enkephalinase. Barker et al. (1989) demonstrated that the CALLA gene, which encodes a 100-kD type II transmembrane glycoprotein, exists in a single copy of greater than 45 kb which is not rearranged in malignancies expressing cell surface CALLA. The gene was located to human chromosome 3 by study of somatic cell hybrids and in situ hybridization regionalized the location to 3q21-q27. Tran-Paterson et al. (1989) also assigned the gene to chromosome 3 by Southern blot analysis of DNA from human-rodent somatic cell hybrids. D'Adamio et al. (1989) demonstrated that the CALLA gene spans more than 80 kb and is composed of 24 exons."

PDE5 Inhibitors

As detailed hereinbefore according to a further embodiment of the present invention there is provided use of a pharmaceutical composition comprising an NEPi and an $I:PDE_{cGMP}$ for use in the treatment of MED. More particularly the present invention provides use of a pharmaceutical composition comprising an NEPi and an $I:PDE5_{cGMP}$ in the manufacture of a medicament for the treatment of MED.

Suitable PDE5i's for use in the pharmaceutical compositions according to the present invention are the cGMP PDE5i's hereinafter detailed. Particularly preferred for use herein are potent and selective cGMP PDE5i's.

Suitable cGMP PDE5 inhibitors for the use according to the present invention include:

the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in EP-A-0463756; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in EP-A-0526004; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in published international patent application WO 93/06104; the isomeric pyrazolo [3,4-d]pyrimidin-4-ones disclosed in published international patent application WO 93/07149; the quinazolin-4-ones disclosed in published international patent application WO 93/12095; the pyrido [3,2-d]pyrimidin-4-ones disclosed in published international patent application WO 94/05661; the purin-6-ones disclosed in published international patent application WO 94/00453; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in published international patent application WO 98/49166; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in published international patent application WO 99/54333; the pyrazolo [4,3-d]pyrimidin-4-ones disclosed in EP-A-0995751; the pyrazolo [4,3-d]pyrimidin-7-ones disclosed in published international patent application WO 00/24745; the pyrazolo [4,3-d]pyrimidin-4-ones disclosed in EP-A-0995750; the compounds disclosed in published international application WO95/19978; the compounds disclosed in published international application WO 99/24433 and the compounds disclosed in published international application WO 93/07124.

It is to be understood that the contents of the above published patent applications, and in particular the general formulae and exemplified compounds therein are incorporated herein in their entirety by reference thereto.

Preferred type V phosphodiesterase inhibitors for the use according to the present invention include:

5-[2-ethoxy-5-(4-methyl-1-piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (sildenafil) also known as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulphonyl]-4-methylpiperazine (see EP-A-0463756);

5-(2-ethoxy-5-morpholinoacetylphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (see EP-A-0526004);

3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-n-propoxyphenyl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (see WO98/49166);

3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (see WO99/54333);

(+)-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxy-1(R)-methylethoxy)pyridin-3-yl]-2-methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, also known as 3-ethyl-5-{5-[4-ethylpiperazin-1-ylsulphonyl]-2-([(1R)-2-methoxy-1-methylethyl]oxy)pyridin-3-yl}-2-methyl-2,6-dihydro-7H-pyrazolo[4,3-d] pyrimidin-7-one (see WO99/54333);

5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, also known as 1-{6-ethoxy-5-[3-ethyl-6,7-dihydro-2-(2-methoxyethyl)-7-oxo-2H-pyrazolo[4,3-d]pyrimidin-5-yl]-3-pyridylsulphonyl}-4-ethylpiperazine (see PDE5 Example 1 hereinafter);

5-[2-*iso*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylpiperidin-4-yl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (see PDE5 Example 2 hereinafter);

5-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-phenyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (see PDE5 Example 3 hereinafter);

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-isopropyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (see PDE5 Example 4 hereinafter);

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (see PDE5 Example 5 hereinafter);

(6R,12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl) -pyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione (IC-351), i.e. the compound of

examples 78 and 95 of published international application WO95/19978, as well as the compound of examples 1, 3, 7 and 8;

2-[2-ethoxy-5-(4-ethyl-piperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one (vardenafil) also known as 1-[[3-(3,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f]-as-triazin-2-yl)-4-ethoxyphenyl]sulphonyl]-4-ethylpiperazine, i.e. the compound of examples 20, 19, 337 and 336 of published international application WO99/24433; and

the compound of example 11 of published international application WO93/07124 (EISAI); and

compounds 3 and 14 from Rotella D P, *J. Med. Chem.,* 2000, 43, 1257.

Still other type cGMP PDE5 inhibitors useful in conjunction with the present invention include:4-bromo-5-(pyridylmethylamino)-6-[3-(4-chlorophenyl)-propoxy]-3(2H)pyridazinone; 1-[4-[(1,3-benzodioxol-5- ylmethyl)amiono]-6-chloro-2-quinozolinyl]-4-piperidine-carboxylic acid, monosodium salt; (+)-cis-5,6a,7,9,9,9a-hexahydro-2-[4-(trifluoromethyl)-phenylmethyl-5-methyl-cyclopent-4,5]imidazo[2,1-b]purin-4(3H)one; furazlocillin; cis-2-hexyl-5-methyl-3,4,5,6a,7,8,9,9a-octahydrocyclopent[4,5]-imidazo[2,1-b]purin-4-one; 3-acetyl-1-(2-chlorobenzyl)-2-propylindole-6- carboxylate; 3-acetyl-1-(2-chlorobenzyl)-2-propylindole-6-carboxylate; 4-bromo-5-(3-pyridylmethylamino)-6-(3-(4-chlorophenyl) propoxy)-3-(2H)pyridazinone; l-methyl-5(5-morpholinoacetyl-2-n-propoxyphenyl)-3-n-propyl-1,6-dihydro- 7H-pyrazolo(4,3-d)pyrimidin-7-one; 1-[4-[(1,3-benzodioxol-5-ylmethyl)arnino]-6-chloro-2- quinazolinyl]-4-piperidinecarboxylic acid, monosodium salt; Pharmaprojects No. 4516 (Glaxo Wellcome); Pharmaprojects No. 5051 (Bayer); Pharmaprojects No. 5064 (Kyowa Hakko; see WO 96/26940); Pharmaprojects No. 5069 (Schering Plough); GF-196960 (Glaxo Wellcome); E-8010 and E-4010 (Eisai); Bay-38-3045 & 38-9456 (Bayer) and Sch-51866.

The suitability of any particular cGMP PDE5 inhibitor can be readily determined by evaluation of its potency and selectivity using literature methods followed by evaluation of its toxicity, absorption, metabolism, pharmacokinetics, etc in accordance with standard pharmaceutical practice.

Preferably, the cGMP PDE5 inhibitors have an IC_{50} at less than 100 nanomolar, more preferably, at less than 50 nanomolar, more preferably still at less than 10 nanomolar.

IC50 values for the cGMP PDE5 inhibitors may be determined using established literature methodology, for example as described in EP0463756-B1 and EP0526004-A1 and as detailed in the Test Methods Section hereinafter.

Preferably the cGMP PDE5 inhibitors used in the pharmaceutical compositions according to the present invention are selective for the PDE5 enzyme. Preferably they are selective over PDE3, more preferably over PDE3 and PDE4. Preferably, the cGMP PDE5 inhibitors of the invention have a selectivity ratio greater than 100 more preferably greater than 300, over PDE3 and more preferably over PDE3 and PDE4.

Selectivity ratios may readily be determined by the skilled person. IC50 values for the PDE3 and PDE4 enzyme may be determined using established literature methodology, see S A Ballard *et al*, Journal of Urology, 1998, vol. 159, pages 2164-2171 and as detailed herein after.

Highly preferred for use in the pharmaceutical compositions herein are potent and selective PDE5 inhibitors.

As discussed hereinbefore the NEPi (and PDE5i where applicable) for use in the treatment of MED in accordance with the present invention may be administered in conjunction with a further pharmaceutically active agent. Here the co-administration need not be done at the same time, let alone by the same route. An example of a co-administration composition could be a composition that comprises an NEPi and an additional agent.

For some applications, the additional agent is an inhibitor – i.e. it is capable of exhibiting an inhibitory function.

For some applications, preferably the additional agent is a NPYi (sometimes written as I:NPY).

For some applications, preferably the additional agent is an NPYi Y1 or NPYi Y2 or NPYi Y5, more preferably the agent is an NPYi Y1.

For some applications, preferably the additional agent is a selective NPYi.

For some applications, preferably the additional agent is an NPYi Y1.

For some applications, preferably the additional agent is a selective NPYi Y1.

TREATMENT

It is to be appreciated that all references herein to treatment include one or more of curative, palliative and prophylactic treatment. Preferably, the term treatment includes at least curative treatment and/or palliative treatment.

SEXUAL STIMULATION

The present invention also encompasses use as defined hereinbefore via administration of a NEPi (and an PDE5i where applicable) before and/or during sexual stimulation. Here the term "sexual stimulation" may be synonymous with the term "sexual arousal". This aspect of the present invention is advantageous because it provides systemic selectivity. The natural cascade only occurs at the genitalia and not in other locations – e.g. in the heart etc. Hence, it would be possible to achieve a selective effect on the genitalia via the MED treatment according to the present invention.

Thus, according to the present invention it is highly desirable that there is a sexual stimulation step at some stage. We have found that this step can provide systemic selectivity. Here, "sexual stimulation" may be one or more of a visual stimulation, a physical stimulation, an auditory stimulation, or a thought stimulation.

AGENT

Agents for use in the treatment of MED according to of the present invention may be any suitable agent that can act as a NEPi and, where appropriate as a PDE5i.

Such agents (i.e. the agents as defined above and/or the additional agents as defined hereinbefore) can be an amino acid sequence or a chemical derivative thereof. The substance may even be an organic compound or other chemical. The agent may even be a nucleotide sequence - which may be a sense sequence or an anti-sense sequence. The agent may even be an antibody.

Thus, the term "agent" includes, but is not limited to, a compound which may be obtainable from or produced by any suitable source, whether natural or not.

The agent may be designed or obtained from a library of compounds which may comprise peptides, as well as other compounds, such as small organic molecules, such as lead compounds.

By way of example, the agent may be a natural substance, a biological macromolecule, or an extract made from biological materials such as bacteria, fungi, or animal (particularly mammalian) cells or tissues, an organic or an inorganic molecule, a synthetic agent, a semi-synthetic agent, a structural or functional mimetic, a peptide, a peptidomimetics, a derivatised agent, a peptide cleaved from a whole protein, or a peptides synthesised synthetically (such as, by way of example, either using a peptide synthesizer or by recombinant techniques or combinations thereof, a recombinant agent, an antibody, a natural or a non-natural agent, a fusion protein or equivalent thereof and mutants, derivatives or combinations thereof.

As used herein, the term "agent" may be a single entity or it may be a combination of agents.

If the agent is an organic compound then for some applications - such as if the agent is a NEPi – that organic compound may typically comprise an amide group (i.e. –N(H)-C(O)- or even –C(O)-N(H)-) and one or more hydrocarbyl groups. Here, the term "hydrocarbyl group" means a group comprising at least C and H and may optionally comprise one or more other suitable substituents. Examples of such substituents may include halo-, alkoxy-, nitro-, an alkyl group, a cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of substituents may form a cyclic group. If the hydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked *via* a suitable element or group. Thus, the hydrocarbyl group may contain hetero atoms. Suitable hetero atoms will

be apparent to those skilled in the art and include, for instance, sulphur, nitrogen and oxygen. For some applications, preferably the agent comprises at least one cyclic group. For some applications, preferably the agent comprises at least one cyclic group linked to another hydrocarbyl group via an amide bond. Examples of such compounds are presented in the Additional Compound Examples section herein.

If the agent is an organic compound then for some applications - such as if the agent is an PDE5i – that organic compound may typically comprise two or more linked hydrocarbyl groups. For some applications, preferably the agent comprises at least two cyclic groups – wherein one of which cyclic groups may be a fused cyclic ring structure. For some applications, preferably at least one of the cyclic groups is a heterocyclic group. For some applications, preferably the heterocyclic group comprises at least one N in the ring. Examples of such compounds are presented in the PDE5 Examples section herein.

If the agent is an organic compound then for some applications - such as if the agent is an I:NPY – that organic compound may typically comprise two or more linked hydrocarbyl groups. For some applications, preferably the agent comprises at least two cyclic groups – optionally wherein one of which cyclic groups may be a fused cyclic ring structure. For some applications, at least one of the cyclic groups is a heterocyclic group. For some applications, preferably the heterocyclic group comprises at least one N in the ring. Examples of such compounds are presented in the Additional Examples section herein.

The agent may contain halo groups. Here, "halo" means fluoro, chloro, bromo or iodo.

The agent may contain one or more of alkyl, alkoxy, alkenyl, alkylene and alkenylene groups – which may be unbranched- or branched-chain.

The agent may be in the form of a pharmaceutically acceptable salt – such as an acid addition salt or a base salt – or a solvate thereof, including a hydrate thereof. For a review on suitable salts see Berge et al, J. Pharm. Sci., 1977, 66, 1-19.

Suitable acid addition salts are formed from acids which form non-toxic salts and examples are the hydrochloride, hydrobromide, hydroiodide, sulphate, bisulphate, nitrate, phosphate, hydrogen phosphate, acetate, maleate, fumarate, lactate, tartrate,

citrate, gluconate, succinate, saccharate, benzoate, methanesulphonate, ethanesulphonate, benzenesulphonate, p-toluenesulphonate and pamoate salts.

Suitable base salts are formed from bases which form non-toxic salts and examples are the sodium, potassium, aluminium, calcium, magnesium, zinc and diethanolamine salts.

A pharmaceutically acceptable salt of an agent as defined hereinbefore may be readily prepared by mixing together solutions of the agent and the desired acid or base, as appropriate. The salt may precipitate from solution and be collected by filtration or may be recovered by evaporation of the solvent.

The agent may exisit in polymorphic form.

The agent may contain one or more asymmetric carbon atoms and therefore exists in two or more stereoisomeric forms. Where an agent contains an alkenyl or alkenylene group, cis (E) and trans (Z) isomerism may also occur. The present invention includes the individual stereoisomers of the agent and, where appropriate, the individual tautomeric forms thereof, together with mixtures thereof.

Separation of diastereoisomers or cis and trans isomers may be achieved by conventional techniques, e.g. by fractional crystallisation, chromatography or H.P.L.C. of a stereoisomeric mixture of the agent or a suitable salt or derivative thereof. An individual enantiomer of the agent may also be prepared from a corresponding optically pure intermediate or by resolution, such as by H.P.L.C. of the corresponding racemate using a suitable chiral support or by fractional crystallisation of the diastereoisomeric salts formed by reaction of the corresponding racemate with a suitable optically active acid or base, as appropriate.

The present invention also includes all suitable isotopic variations of the agent or a pharmaceutically acceptable salt thereof. An isotopic variation of an agent of the present invention or a pharmaceutically acceptable salt thereof is defined as one in which at least one atom is replaced by an atom having the same atomic number but an atomic mass different from the atomic mass usually found in nature. Examples of isotopes that can be incorporated into the agent and pharmaceutically acceptable salts thereof include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulphur, fluorine and chlorine such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F

and ^{36}Cl, respectively. Certain isotopic variations of the agent and pharmaceutically acceptable salts thereof, for example, those in which a radioactive isotope such as ^{3}H or ^{14}C is incorporated, are useful in drug and/or substrate tissue distribution studies. Tritiated, i.e., ^{3}H, and carbon-14, i.e., ^{14}C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with isotopes such as deuterium, i.e., ^{2}H, may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements and hence may be preferred in some circumstances. Isotopic variations of the agent and pharmaceutically acceptable salts thereof can generally be prepared by conventional procedures using appropriate isotopic variations of suitable reagents.

It will be appreciated by those skilled in the art that the agent may be derived from a prodrug. Examples of prodrugs include entities that have certain protected group(s) and which may not possess pharmacological activity as such, but may, in certain instances, be administered (such as orally or parenterally) and thereafter metabolised in the body to form the agent which are pharmacologically active.

It will be further appreciated that certain moieties known as "pro-moieties", for example as described in "Design of Prodrugs" by H. Bundgaard, Elsevier, 1985 (the disclosured of which is hereby incorporated by reference), may be placed on appropriate functionalities of the agents. Such prodrugs are also included within the scope of the invention.

The term inhibitor as used herein in relation to the NEPi and PDE5i compounds is to be regarded as being interchangeable with the term antagonist. Further the phrase, enhancing the endogenous erectile process, is to be regarded as being interchangeable with the phrase upregulation of the endogenous erectile process.

For some applications (such as a topical application), the agent may also display an ACE (angiotensin converting enzyme) inhibitory action. An ACE assay is presented in the Experimental Section herein. For some applications (such as with particular patient types), such agents (i.e. those that also display ACE inhibitory action) may not be suitable for oral administration.

For some applications, the agent may also display an ECE (endothelium converting enzyme) inhibitory action. ECE assays are well known in the art.

PHARMACEUTICAL COMBINATIONS

As discussed hereinbefore treatment of MED according to the present invention may be achieved via a combination of a NEPi with one or more other additional pharmaceutically active agents, such as a nitric oxide donor, or a nitric oxide precursor eg L-arginine or inhibitors of arginase) and/or a centrally acting pharmaceutical (e.g. a dopamine receptor agonist such as apomorphine or selective dopamine D2 receptor agonists such as PNU-95666 or a melanocortin receptor agonist, such as melanotan II). Teachings on the use of apomorphine as a pharmaceutical may be found in US-A-5945117. In that particular document, apomorphine is delivered sub-lingually. In addition, or in the alternative, the agent may be used in combination with one or more of: one or more of a nitric oxide donor (eg NMI-921), one or more of a dopamine receptor agonist (eg apomorphine, Uprima, Ixsene), one or more of a heterocyclic amine such as generically and specifically disclosed in WO 00/40226, in particular example numbers 7, 8 and 9, one or more of a melanocortin receptor agonist (eg Melanotan II or PT14), one or more of a potassium channel opener (eg a K_{ATP} channel opener (eg minoxidil, nicorandil) and/or a calcium activated potassium channel opener (eg BMS-204352), one or more of an α1-adrenoceptor antagonist (eg phentolamine, Vasomax), one or more of a VIP receptor agonist or a VIP analogue (eg Ro-125-1553) or a VIP fragment, one or more of a α-adrenoceptor antagonist with VIP combination (eg Invicorp, Aviptadil), one or more of a α2-adrenoceptor antagonist (eg yohimbine), one or more of a testosterone replacement agent (inc DHEA (dehydroandrostendione), testosterone (Tostrelle) or a testosterone implant (Organon)), one or more of a testosterone/oestradiol agent one or more of an estrogen agonists eg Lasofoxifene, one or more of a serotonin receptor agonist or antagonist (eg 5HT1A, 5HT2C, 5HT2A and 5HT3 receptor agonists and antagonists; as described in WO2000/28993), one or more of a prostanoid receptor agonist (eg Muse, alprostadil, misoprostol), one or more of a purinergic receptor agonist (especially P2Y2 and P2Y4) one or more antidepressant agents (eg bupropion (Wellbutrin), mirrtazapine, nefazodone).

If a combination of additional active agents are administered, then they may be administered simultaneously, separately or sequentially with or to the NEPi as detailed hereinbefore.

Pharmaceutical Formulations

The compounds of the invention, their pharmaceutically acceptable salts, and pharmaceutically acceptable solvates of either entity can be administered alone but, in human therapy will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

For example, the compounds of the invention, or salts or solvates thereof can be administered orally, buccally or sublingually in the form of tablets, capsules (including soft gel capsules), ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed-, modified-, or controlled-release such as sustained-, dual-, or pulsatile delivery applications. The compounds of the invention may also be administered via intracavernosal injection. The compounds of the invention may also be administered via fast dispersing or fast dissolving dosages forms or in the form of a high energy dispersion or as coated particles. Suitable pharmaceutical formulations of the compounds of the invention may be in coated or un-coated form as desired.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate, glycine and starch (preferably corn, potato or tapioca starch), disintegrants such as sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethyl cellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

Modified release and pulsatile release dosage forms may contain excipients such as those detailed for immediate release dosage forms together with additional excipients that act as release rate modifiers, these being coated on and/or included in the body of the device. Release rate modifiers include, but are not exclusively limited to, hydroxypropylmethyl cellulose, methyl cellulose, sodium carboxymethylcellulose, ethyl cellulose, cellulose acetate, polyethylene oxide, Xanthan gum, Carbomer, ammonio methacrylate copolymer, hydrogenated castor oil, carnauba wax, paraffin wax, cellulose acetate phthalate, hydroxypropylmethyl cellulose phthalate, methacrylic acid copolymer and mixtures thereof. Modified release and pulsatile release dosage forms may contain one or a combination of release rate modifying excipients. Release rate modifying excipients maybe present both within the dosage form i.e. within the matrix, and/or on the dosage form i.e. upon the surface or coating.

Fast dispersing or dissolving dosage formulations (FDDFs) may contain the following ingredients: aspartame, acesulfame potassium, citric acid, croscarmellose sodium, crospovidone, diascorbic acid, ethyl acrylate, ethyl cellulose, gelatin, hydroxypropylmethyl cellulose, magnesium stearate, mannitol, methyl methacrylate, mint flavouring, polyethylene glycol, fumed silica, silicon dioxide, sodium starch glycolate, sodium stearyl fumarate, sorbitol, xylitol. The terms dispersing or dissolving as used herein to describe FDDFs are dependent upon the solubility of the drug substance used i.e. where the drug substance is insoluble a fast dispersing dosage form can be prepared and where the drug substance is soluble a fast dissolving dosage form can be prepared.

The compounds of the invention can also be administered parenterally, for example, intracavernosally, intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intraurethrally intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion or needless injection techniques. For such parenteral administration they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

For oral and parenteral administration to human patients, the daily dosage level of the compounds of the invention or salts or solvates thereof will usually be from 10 to 500 mg (in single or divided doses).

Thus, for example, tablets or capsules of the compounds of the invention or salts or solvates thereof may contain from 5 mg to 250 mg of active compound for administration singly or two or more at a time, as appropriate. The physician in any event will determine the actual dosage which will be most suitable for any individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited and such are within the scope of this invention. The skilled person will also appreciate that, for in the treatment of MED according to the present invention, the NEPi (and where appropriate PDE5i or additional agents(s)) compounds may be taken as a single dose on an "as required" basis (i.e. as needed or desired).

Example Tablet Formulation

In general a tablet formulation could typically contain between about 0.01mg and 500mg of compound (or a salt thereof) whilst tablet fill weights may range from 50mg to 1000mg. An example formulation for a 10mg tablet is illustrated:

Ingredient	%w/w
Free acid, Free base or Salt of Compound	10.000*
Lactose	64.125
Starch	21.375
Croscarmellose Sodium	3.000
Magnesium Stearate	1.500

* This quantity is typically adjusted in accordance with drug activity.

The tablets are manufactured by a standard process, for example, direct compression or a wet or dry granulation process. The tablet cores may be coated with appropriate overcoats.

The compounds / compositions can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A [trade mark] or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA [trade mark]), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active compound, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains from 1 to 50 mg of a compound of the invention for delivery to the patient. The overall daily dose with an aerosol will be in the range of from 1 to 50 mg which may be administered in a single dose or, more usually, in divided doses throughout the day.

The compounds also be formulated for delivery via an atomiser. Formulations for atomiser devices may contain the following ingredients as solubilisers, emulsifiers or suspending agents: water, ethanol, glycerol, propylene glycol, low molecular weight polyethylene glycols, sodium chloride, fluorocarbons, polyethylene glycol ethers, sorbitan trioleate, oleic acid.

Alternatively, the compounds or salts or solvates thereof can be administered in the form of a suppository, or they may be applied topically in the form of a gel, hydrogel, lotion, solution, cream, ointment or dusting powder. The compounds of the invention or salts or solvates thereof may also be dermally administered. The compounds of the invention or salts or solvates thereof may also be transdermally administered, for example, by the use of a skin patch. They may also be administered by the ocular, pulmonary or rectal routes.

For ophthalmic use, the compounds can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH

adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds or salts or solvates thereof can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

The compounds may also be used in combination with a cyclodextrin. Cyclodextrins are known to form inclusion and non-inclusion complexes with drug molecules. Formation of a drug-cyclodextrin complex may modify the solubility, dissolution rate, bioavailability and/or stability property of a drug molecule. Drug-cyclodextrin complexes are generally useful for most dosage forms and administration routes. As an alternative to direct complexation with the drug the cyclodextrin may be used as an auxiliary additive, e.g. as a carrier, diluent or solubiliser. Alpha-, beta- and gamma-cyclodextrins are most commonly used and suitable examples are described in WO-A-91/11172, WO-A-94/02518 and WO-A-98/55148.

Generally, in humans, oral administration of the is the preferred route, being the most convenient in MED, avoiding the well-known disadvantages associated with intracavernosal (i.c.) administration. A preferred oral dosing regimen in MED for a typical man is from about 25mg to 500 mg of pharmaceutical composition when required. Where the composition comprises the combination of a NEPi and a PDE5I then from 25mg to 250mg of each compound may be present. In circumstances where the recipient suffers from a swallowing disorder or from impairment of drug absorption after oral administration, the drug may be administered parenterally, sublingually or buccally.

<u>K_i VALUES</u>

For some applications, preferably the agent of the present invention (and optionally the optional additional agent) has a K_i value of less than about 100 nM, preferably less than about 75 nM, preferably less than about 50 nM, preferably less than about 25 nM, preferably less than about 20 nM, preferably less than about 15 nM, preferably less than about 10 nM, preferably less than about 5 nM.

PHARMACOKINETICS

For some embodiments of the present invention, preferably the NEPi agents for use in the treatment of MED according to the present invention (and optionally the optional additional agent) have a log D of -2 to +4, more preferably -1 to +2. The log D can be determined by standard procedures known in the art such as described in J. Pharm. Pharmacol. 1990, 42:144.

In addition, or in the alternative to the above, for some embodiments preferably the NEPi agents (and optionally the PDE5i and/or optional additional agent(s)) have a caco-2 flux of greater than $2x10^{-6}cms^{-1}$, more preferably greater than $5x10^{-6}cms^{-1}$. The caco flux value can be determined by standard procedures known in the art such as described in J. Pharm. Sci 79, 7, p595-600 (1990), and Pharm. Res. vol 14, no. 6 (1997).

CHEMICAL SYNTHESIS METHODS

Typically the NEPi and PDE5i compounds suitable for the use according to the present invention will be prepared by chemical synthesis techniques.

The agent or target or variants, homologues, derivatives, fragments or mimetics thereof may be produced using chemical methods to synthesize the agent in whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (e.g., Creighton (1983) Proteins Structures And Molecular Principles, WH Freeman and Co, New York NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, *supra*).

Direct synthesis of the agent or variants, homologues, derivatives, fragments or mimetics thereof can be performed using various solid-phase techniques (Roberge JY *et al* (1995) Science 269: 202-204) and automated synthesis may be achieved, for example, using the ABI 43 1 A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer. Additionally, the amino acid sequences comprising the agent or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with a sequence from other subunits, or any part thereof, to produce a variant agent or target, such as, for example, a variant NEP.

In an alternative embodiment of the invention, the coding sequence of the agent target or variants, homologues, derivatives, fragments or mimetics thereof may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers MH *et al* (1980) Nuc Acids Res Symp Ser 215-23, Horn T *et al* (1980) Nuc Acids Res Symp Ser 225-232).

MIMETIC

As used herein, the term "mimetic" relates to any chemical which includes, but is not limited to, a peptide, polypeptide, antibody or other organic chemical which has the same qualitative activity or effect as a reference agent to a target.

CHEMICAL DERIVATIVE

The term "derivative" or “derivatised” as used herein includes chemical modification of an agent. Illustrative of such chemical modifications would be replacement of hydrogen by a halo group, an alkyl group, an acyl group or an amino group.

CHEMICAL MODIFICATION

In one embodiment of the present invention, the agent may be a chemically modified agent.

The chemical modification of an agent may either enhance or reduce hydrogen bonding interaction, charge interaction, hydrophobic interaction, Van Der Waals interaction or dipole interaction between the agent and the target.

In one aspect, the identified agent may act as a model (for example, a template) for the development of other compounds.

RECOMBINANT METHODS

Typically the target for use in the assay of the present invention may be prepared by recombinant DNA techniques.

AMINO ACID SEQUENCE

As used herein, the term "amino acid sequence" is synonymous with the term "polypeptide" and/or the term "protein". In some instances, the term "amino acid sequence" is synonymous with the term "peptide". In some instances, the term "amino acid sequence" is synonymous with the term "protein".

The amino acid sequence may be prepared isolated from a suitable source, or it may be made synthetically or it may be prepared by use of recombinant DNA techniques.

NUCLEOTIDE SEQUENCE

As used herein, the term "nucleotide sequence" is synonymous with the term "polynucleotide".

The nucleotide sequence may be DNA or RNA of genomic or synthetic or of recombinant origin. The nucleotide sequence may be double-stranded or single-stranded whether representing the sense or antisense strand or combinations thereof.

For some applications, preferably, the nucleotide sequence is DNA.

For some applications, preferably, the nucleotide sequence is prepared by use of recombinant DNA techniques (e.g. recombinant DNA).

For some applications, preferably, the nucleotide sequence is cDNA.

For some applications, preferably, the nucleotide sequence may be the same as the naturally occurring form for this aspect.

It will be understood by a skilled person that numerous different nucleotide sequences can encode the targets as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not substantially affect the activity encoded by the nucleotide sequence of the present invention to reflect the codon usage of any particular host organism in which the target is to be expressed. Thus, the terms "variant", "homologue" or "derivative" in relation to the nucleotide sequence set out in the attached sequence listings include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence encodes a functional target according the present invention (or even an agent according to the present invention if said agent comprises a nucleotide sequence or an amino acid sequence).

As indicated above, with respect to sequence homology, preferably there is at least 75%, more preferably at least 85%, more preferably at least 90% homology to the sequences shown in the sequence listing herein. More preferably there is at least 95%, more preferably at least 98%, homology. Nucleotide homology comparisons may be conducted as described above. A preferred sequence comparison program is the GCG Wisconsin Bestfit program described above. The default scoring matrix has a match value of 10 for each identical nucleotide and -9 for each mismatch. The default gap creation penalty is -50 and the default gap extension penalty is -3 for each nucleotide.

The present invention also encompasses nucleotide sequences that are capable of hybridising selectively to the sequences presented herein, or any variant, fragment or derivative thereof, or to the complement of any of the above. Nucleotide sequences are preferably at least 15 nucleotides in length, more preferably at least 20, 30, 40 or 50 nucleotides in length. These sequences could be used a probes, such as in a diagnostic kit.

VARIANTS/HOMOLOGUES/DERIVATIVES

In addition to the specific amino acid sequences and nucleotide sequences mentioned herein, the present invention also encompasses the use of variants;

homologue and derivatives thereof. Here, the term "homology" can be equated with "identity".

In the present context, an homologous sequence is taken to include an amino acid sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98% identical. In particular, homology should typically be considered with respect to those regions of the sequence known to be essential for an activity. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce

optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package (see below) the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux *et al.,* 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel *et al.*, 1999 *ibid* – Chapter 18), FASTA (Atschul *et al.,* 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel *et al.*, 1999 *ibid*, pages 7-58 to 7-60). However it is preferred to use the GCG Bestfit program. A new tool, called BLAST 2 Sequences is also available for comparing protein and nucleotide sequence (see FEMS Microbiol Lett 1999 174(2): 247-50; FEMS Microbiol Lett 1999 177(1): 187-8 and tatiana@ncbi.nlm.nih.gov).

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). It is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

The sequences may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent substance. Deliberate amino acid substitutions may be made on the basis of

similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the secondary binding activity of the substance is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

The present invention also encompasses homologous substitution (substitution and replacement are both used herein to mean the interchange of an existing amino acid residue, with an alternative residue) may occur i.e. like-for-like substitution such as basic for basic, acidic for acidic, polar for polar etc. Non-homologous substitution may also occur i.e. from one class of residue to another or alternatively involving the inclusion of unnatural amino acids such as ornithine (hereinafter referred to as Z), diaminobutyric acid ornithine (hereinafter referred to as B), norleucine ornithine (hereinafter referred to as O), pyriylalanine, thienylalanine, naphthylalanine and phenylglycine.

Replacements may also be made by unnatural amino acids include; alpha* and alpha-disubstituted* amino acids, N-alkyl amino acids*, lactic acid*, halide derivatives of natural amino acids such as trifluorotyrosine*, p-Cl-phenylalanine*, p-Br-phenylalanine*, p-I-phenylalanine*, L-allyl-glycine*, ß-alanine*, L-α-amino butyric acid*, L-γ-amino butyric acid*, L-α-amino isobutyric acid*, L-ε-amino caproic acid#, 7-amino heptanoic acid*, L-methionine sulfone#*, L-norleucine*, L-norvaline*, p-nitro-L-phenylalanine*, L-hydroxyproline#, L-thioproline*, methyl derivatives of phenylalanine (Phe) such as 4-methyl-Phe*, pentamethyl-Phe*, L-Phe (4-amino)#, L-Tyr (methyl)*,

L-Phe (4-isopropyl)*, L-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxyl acid)*, L-diaminopropionic acid# and L-Phe (4-benzyl)*. The notation * has been utilised for the purpose of the discussion above (relating to homologous or non-homologous substitution), to indicate the hydrophobic nature of the derivative whereas # has been utilised to indicate the hydrophilic nature of the derivative, #* indicates amphipathic characteristics.

Variant amino acid sequences may include suitable spacer groups that may be inserted between any two amino acid residues of the sequence including alkyl groups such as methyl, ethyl or propyl groups in addition to amino acid spacers such as glycine or β-alanine residues. A further form of variation, involves the presence of one or more amino acid residues in peptoid form, will be well understood by those skilled in the art. For the avoidance of doubt, "the peptoid form" is used to refer to variant amino acid residues wherein the α-carbon substituent group is on the residue's nitrogen atom rather than the α-carbon. Processes for preparing peptides in the peptoid form are known in the art, for example Simon RJ et al., PNAS (1992) 89(20), 9367-9371 and Horwell DC, Trends Biotechnol. (1995) 13(4), 132-134.

HYBRIDISATION

The present invention also encompasses the use of sequences that can hybridise to the target sequences presented herein – such as if the agent is an anti-sense sequence.

The term "hybridization" as used herein shall include "the process by which a strand of nucleic acid joins with a complementary strand through base pairing" as well as the process of amplification as carried out in polymerase chain reaction (PCR) technologies.

Nucleotide sequences of the invention capable of selectively hybridising to the nucleotide sequences presented herein, or to their complement, will be generally at least 75%, preferably at least 85 or 90% and more preferably at least 95% or 98% homologous to the corresponding complementary nucleotide sequences presented herein over a region of at least 20, preferably at least 25 or 30, for instance at least 40, 60 or 100 or more contiguous nucleotides. Preferred nucleotide sequences of the invention will comprise regions homologous to the nucleotide sequence set out in SEQ

ID No 2 of the sequence listings of the present invention preferably at least 80 or 90% and more preferably at least 95% homologous to the nucleotide sequence set out in SEQ ID No 2 of the sequence listings of the present invention.

The term "selectively hybridizable" means that the nucleotide sequence, when used as a probe, is used under conditions where a target nucleotide sequence is found to hybridize to the probe at a level significantly above background. The background hybridization may occur because of other nucleotide sequences present, for example, in the cDNA or genomic DNA library being screened. In this event, background implies a level of signal generated by interaction between the probe and a non-specific DNA member of the library which is less than 10 fold, preferably less than 100 fold as intense as the specific interaction observed with the target DNA. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ^{32}P.

Hybridization conditions are based on the melting temperature (Tm) of the nucleic acid binding complex, as taught in Berger and Kimmel (1987, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol 152, Academic Press, San Diego CA), and confer a defined "stringency" as explained below.

Maximum stringency typically occurs at about Tm-5°C (5°C below the Tm of the probe); high stringency at about 5°C to 10°C below Tm; intermediate stringency at about 10°C to 20°C below Tm; and low stringency at about 20°C to 25°C below Tm. As will be understood by those of skill in the art, a maximum stringency hybridization can be used to identify or detect identical nucleotide sequences while an intermediate (or low) stringency hybridization can be used to identify or detect similar or related polynucleotide sequences.

In a preferred aspect, the present invention covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention under stringent conditions (e.g. 65°C and 0.1xSSC {1xSSC = 0.15 M NaCl, 0.015 M Na_3 Citrate pH 7.0). Where the nucleotide sequence of the invention is double-stranded, both strands of the duplex, either individually or in combination, are encompassed by the present invention. Where the nucleotide sequence is single-stranded, it is to be understood that the complementary sequence of that nucleotide sequence is also included within the scope of the present invention.

Nucleotide sequences which are not 100% homologous to the sequences of the present invention but fall within the scope of the invention can be obtained in a number of ways. Other variants of the sequences described herein may be obtained for example by probing DNA libraries made from a range of sources. In addition, other viral/bacterial, or cellular homologues particularly cellular homologues found in mammalian cells (e.g. rat, mouse, bovine and primate cells), may be obtained and such homologues and fragments thereof in general will be capable of selectively hybridising to the sequences shown in the sequence listing herein. Such sequences may be obtained by probing cDNA libraries made from or genomic DNA libraries from other animal species, and probing such libraries with probes comprising all or part of the nucleotide sequence set out in herein under conditions of medium to high stringency. Similar considerations apply to obtaining species homologues and allelic variants of the amino acid and/or nucleotide sequences of the present invention.

Variants and strain/species homologues may also be obtained using degenerate PCR which will use primers designed to target sequences within the variants and homologues encoding conserved amino acid sequences within the sequences of the present invention. Conserved sequences can be predicted, for example, by aligning the amino acid sequences from several variants/homologues. Sequence alignments can be performed using computer software known in the art. For example the GCG Wisconsin PileUp program is widely used. The primers used in degenerate PCR will contain one or more degenerate positions and will be used at stringency conditions lower than those used for cloning sequences with single sequence primers against known sequences.

Alternatively, such nucleotide sequences may be obtained by site directed mutagenesis of characterised sequences, such as the nucleotide sequence set out in SEQ ID No 2 of the sequence listings of the present invention. This may be useful where for example silent codon changes are required to sequences to optimise codon preferences for a particular host cell in which the nucleotide sequences are being expressed. Other sequence changes may be desired in order to introduce restriction enzyme recognition sites, or to alter the activity of the protein encoded by the nucleotide sequences.

The nucleotide sequences of the present invention may be used to produce a primer, e.g. a PCR primer, a primer for an alternative amplification reaction, a probe e.g. labelled with a revealing label by conventional means using radioactive or non-radioactive labels, or the nucleotide sequences may be cloned into vectors. Such

primers, probes and other fragments will be at least 15, preferably at least 20, for example at least 25, 30 or 40 nucleotides in length, and are also encompassed by the term nucleotide sequence of the invention as used herein.

The nucleotide sequences such as a DNA polynucleotides and probes according to the invention may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques.

In general, primers will be produced by synthetic means, involving a step wise manufacture of the desired nucleic acid sequence one nucleotide at a time. Techniques for accomplishing this using automated techniques are readily available in the art.

Longer nucleotide sequences will generally be produced using recombinant means, for example using a PCR (polymerase chain reaction) cloning techniques. This will involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking a region of the targeting sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction (PCR) under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector.

Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used to clone and express the target sequences. As will be understood by those of skill in the art, for certain expression systems, it may be advantageous to produce the target sequences with non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host (Murray E *et al* (1989) Nuc Acids Res 17:477-508) can be selected, for example, to increase the rate of the target expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

EXPRESSION VECTORS

The nucleotide sequence for use as the target or for expressing the target can be incorporated into a recombinant replicable vector. The vector may be used to replicate and express the nucleotide sequence in and/or from a compatible host cell. Expression may be controlled using control sequences which include promoters/enhancers and other expression regulation signals. Prokaryotic promoters and promoters functional in eukaryotic cells may be used. Tissue specific or stimuli specific promoters may be used. Chimeric promoters may also be used comprising sequence elements from two or more different promoters described above.

The protein produced by a host recombinant cell by expression of the nucleotide sequence may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. The coding sequences can be designed with signal sequences which direct secretion of the substance coding sequences through a particular prokaryotic or eukaryotic cell membrane.

FUSION PROTEINS

The target amino acid sequence may be produced as a fusion protein, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), 6xHis, GAL4 (DNA binding and/or transcriptional activation domains) and β-galactosidase. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to allow removal of fusion protein sequences. Preferably the fusion protein will not hinder the activity of the target.

The fusion protein may comprise an antigen or an antigenic determinant fused to the substance of the present invention. In this embodiment, the fusion protein may be a non-naturally occurring fusion protein comprising a substance which may act as an adjuvant in the sense of providing a generalised stimulation of the immune system. The antigen or antigenic determinant may be attached to either the amino or carboxy terminus of the substance.

In another embodiment of the invention, the amino acid sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for agents capable of affecting the substance activity, it may be useful to encode a chimeric substance expressing a heterologous epitope that is recognized by a commercially available antibody.

ANTIBODIES

In one embodiment of the present invention, the agent may be an antibody. In addition, or in the alternative, the target may be an antibody. In addition, or in the alternative, the means for detecting the target may be an antibody.

Antibodies may be produced by standard techniques, such as by immunisation with the substance of the invention or by using a phage display library.

For the purposes of this invention, the term "antibody", unless specified to the contrary, includes but is not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments, fragments produced by a Fab expression library, as well as mimetics thereof. Such fragments include fragments of whole antibodies which retain their binding activity for a target substance, Fv, F(ab') and $F(ab')_2$ fragments, as well as single chain antibodies (scFv), fusion proteins and other synthetic proteins which comprise the antigen-binding site of the antibody. Furthermore, the antibodies and fragments thereof may be humanised antibodies. Neutralizing antibodies; i.e., those which inhibit biological activity of the substance polypeptides, are especially preferred for diagnostics and therapeutics.

If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunised with an immunogenic polypeptide bearing a epitope(s) obtainable from an identified agent and/or substance of the present invention. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminium hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (*Bacilli Calmette-Guerin*) and *Corynebacterium parvum* are potentially useful human adjuvants which may be employed if purified the substance polypeptide is administered to immunologically compromised individuals for the purpose of stimulating systemic defence.

Serum from the immunised animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to an epitope obtainable from an identifed agent and/or substance of the present invention contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity

chromatography. Techniques for producing and processing polyclonal antisera are known in the art. In order that such antibodies may be made, the invention also provides polypeptides of the invention or fragments thereof haptenised to another polypeptide for use as immunogens in animals or humans.

Monoclonal antibodies directed against epitopes obtainable from an identifed agent and/or substance of the present invention can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. Panels of monoclonal antibodies produced against orbit epitopes can be screened for various properties; i.e., for isotype and epitope affinity.

Monoclonal antibodies to the substance and/or identified agent may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique originally described by Koehler and Milstein (1975 Nature 256:495-497), the human B-cell hybridoma technique (Kosbor *et al* (1983) Immunol Today 4:72; Cote *et al* (1983) Proc Natl Acad Sci 80:2026-2030) and the EBV-hybridoma technique (Cole *et al* (1985) Monoclonal Antibodies and Cancer Therapy, Alan R Liss Inc, pp 77-96). In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison *et al* (1984) Proc Natl Acad Sci 81:6851-6855; Neuberger *et al* (1984) Nature 312:604-608; Takeda *et al* (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies (US Patent No. 4,946,779) can be adapted to produce the substance specific single chain antibodies.

Antibodies, both monoclonal and polyclonal, which are directed against epitopes obtainable from an identifed agent and/or substance are particularly useful in diagnosis, and those which are neutralising are useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotype antibodies. Anti-idiotype antibodies are immunoglobulins which carry an "internal image" of the substance and/or agent against which protection is desired. Techniques for raising anti-idiotype antibodies are known in the art. These anti-idiotype antibodies may also be useful in therapy.

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi *et al* (1989, Proc Natl Acad Sci 86: 3833-3837), and Winter G and Milstein C (1991; Nature 349:293-299).

Antibody fragments which contain specific binding sites for the substance may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse WD *et al* (1989) Science 256:1275-128 1).

REPORTERS

A wide variety of reporters may be used in the assay methods (as well as screens) of the present invention with preferred reporters providing conveniently detectable signals (eg. by spectroscopy). By way of example, a reporter gene may encode an enzyme which catalyses a reaction which alters light absorption properties.

Examples of reporter molecules include but are not limited to β-galactosidase, invertase, green fluorescent protein, luciferase, chloramphenicol, acetyltransferase, β-glucuronidase, exo-glucanase and glucoamylase. Alternatively, radiolabelled or fluorescent tag-labelled nucleotides can be incorporated into nascent transcripts which are then identified when bound to oligonucleotide probes.

In one preferred embodiment, the production of the reporter molecule is measured by the enzymatic activity of the reporter gene product, such as β-galactosidase.

A variety of protocols for detecting and measuring the expression of the target, such as by using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilising monoclonal antibodies reactive to two non-interfering epitopes on polypeptides is preferred, but a competitive binding assay

may be employed. These and other assays are described, among other places, in Hampton R *et al* (1990, Serological Methods, A Laboratory Manual, APS Press, St Paul MN) and Maddox DE *et al* (1983, J Exp Med 15 8:121 1).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic and amino acid assays. Means for producing labelled hybridisation or PCR probes for detecting the target polynucleotide sequences include oligolabelling, nick translation, end-labelling or PCR amplification using a labelled nucleotide. Alternatively, the coding sequence, or any portion of it, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labelled nucleotides.

A number of companies such as Pharmacia Biotech (Piscataway, NJ), Promega (Madison, WI), and US Biochemical Corp (Cleveland, OH) supply commercial kits and protocols for these procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles and the like. Patents teaching the use of such labels include US-A-3817837; US-A-3850752; US-A-3939350; US-A-3996345; US-A-4277437; US-A-4275149 and US-A-4366241. Also, recombinant immunoglobulins may be produced as shown in US-A-4816567.

Additional methods to quantify the expression of a particular molecule include radiolabeling (Melby PC *et al* 1993 J Immunol Methods 159:235-44) or biotinylating (Duplaa C *et al* 1993 Anal Biochem 229-36) nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated. Quantification of multiple samples may be speeded up by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or calorimetric response gives rapid quantification.

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression should be confirmed. For example, if the nucleotide sequence is inserted within a marker gene sequence, recombinant cells containing the same may be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a target coding sequence under the control of a single promoter. Expression of the marker

gene in response to induction or selection usually indicates expression of the target as well.

Alternatively, host cells which contain the coding sequence for the target and express the target coding regions may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridisation and protein bioassay or immunoassay techniques which include membrane-based, solution-based, or chip-based technologies for the detection and/or quantification of the nucleic acid or protein.

SCREENS

Any one or more of appropriate targets - such as an amino acid sequence and/or nucleotide sequence - may be used for identifying a NEPi in any of a variety of drug screening techniques. The target employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The target may even be within an animal model, wherein said target may be an exogenous target or an introduced target. The animal model will be a non-human animal model. The abolition of target activity or the formation of binding complexes between the target and the agent being tested may be measured.

Techniques for drug screening may be based on the method described in Geysen, European Patent Application 84/03564, published on September 13, 1984. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with a suitable target or fragment thereof and washed. Bound entities are then detected - such as by appropriately adapting methods well known in the art. A purified target can also be coated directly onto plates for use in a drug screening techniques. Alternatively, non-neutralising antibodies can be used to capture the peptide and immobilise it on a solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralising antibodies capable of binding a target specifically compete with a test compound for binding to a target.

Another technique for screening provides for high throughput screening (HTS) of agents having suitable binding affinity to the substances and is based upon the method described in detail in WO 84/03564.

It is expected that the assay methods of the present invention will be suitable for both small and large-scale screening of test compounds as well as in quantitative assays.

In a preferred aspect, the screen of the present invention comprises at least the following steps (which need not be in this same consecutive order): (a) conducting an *in vitro* screen to determine whether a candidate agent has the relevant activity (such as modulation of NEP, such as NEP from dog kidney); (b) conducting one or more selectivity screens to determine the selectivity of said candidate agent (e.g. to see if said agent is also an ACE inhibitor – such as by using the assay protocol presented herein); and (c) conducting an *in vivo* screen with said candidate agent (e.g. using a functional animal model). Typically, if said candidate agent passes screen (a) and screen (b) then screen (c) is performed.

DIAGNOSTICS

The present invention also provides a diagnostic composition or kit for the detection of a pre-disposition for MED. In this respect, the composition or kit will comprise an entity that is capable of indicating the presence of one or more - or even the absence of one or more - of the targets in a test sample. Preferably, the test sample is obtained from the penis.

By way of example, the diagnostic composition may comprise any one of the nucleotide sequences mentioned herein or a variant, homologue, fragment or derivative thereof, or a sequence capable of hybridising to all or part of any one of the nucleotide sequence.

In order to provide a basis for the diagnosis of disease, normal or standard values from a target should be established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with an antibody to a target under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by comparing it to a dilution series of positive controls where a known amount of antibody is combined with known concentrations of a purified target. Then, standard

values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by MED. Deviation between standard and subject values establishes the presence of the disease state.

A target itself, or any part thereof, may provide the basis for a diagnostic and/or a therapeutic compound. For diagnostic purposes, target polynucleotide sequences may be used to detect and quantify gene expression in conditions, disorders or diseases in which MED may be implicated.

The target encoding polynucleotide sequence may be used for the diagnosis of MED resulting from expression of the target. For example, polynucleotide sequences encoding a target may be used in hybridisation or PCR assays of tissues from biopsies or autopsies or biological fluids, to detect abnormalities in target expression. The form of such qualitative or quantitative methods may include Southern or northern analysis, dot blot or other membrane-based technologies; PCR technologies; dip stick, pin or chip technologies; and ELISA or other multiple sample formal technologies. All of these techniques are well known in the art and are in fact the basis of many commercially available diagnostic kits.

Such assays may be tailored to evaluate the efficacy of a particular therapeutic treatment regime and may be used in animal studies, in clinical trials, or in monitoring the treatment of an individual patient. In order to provide a basis for the diagnosis of disease, a normal or standard profile for target expression should be established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with the target or a portion thereof, under conditions suitable for hybridisation or amplification. Standard hybridisation may be quantified by comparing the values obtained for normal subjects with a dilution series of positive controls run in the same experiment where a known amount of purified target is used. Standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by a disorder or disease related to expression of the target coding sequence. Deviation between standard and subject values establishes the presence of the disease state. If disease is established, an existing therapeutic agent is administered, and treatment profile or values may be generated. Finally, the assay may be repeated on a regular basis to evaluate whether the values progress toward or return to the normal or standard pattern. Successive treatment profiles may be used to show the efficacy of treatment over a period of several days or several months.

Thus, in one aspect, the present invention relates to the use of a target polypeptide, or variant, homologue, fragment or derivative thereof, to produce anti-target antibodies which can, for example, be used diagnostically to detect and quantify target levels in MED.

The present invention further provides diagnostic assays and kits for the detection of a target in cells and tissues comprising a purified target which may be used as a positive control, and anti-target antibodies. Such antibodies may be used in solution-based, membrane-based, or tissue-based technologies to detect any disease state or condition related to the expression of target protein or expression of deletions or a variant, homologue, fragment or derivative thereof.

ASSAY METHODS

The diagnostic compositions and/or methods and/or kits may be used in the following techniques which include but are not limited to; competitive and non-competitive assays, radioimmunoassay, bioluminescence and chemiluminescence assays, fluorometric assays, sandwich assays, immunoradiometric assays, dot blots, enzyme linked assays including ELISA, microtiter plates, antibody coated strips or dipsticks for rapid monitoring of urine or blood, immunohistochemistry and immunocytochemistry.

By way of example, an immunohistochemistry kit may also be used for localization of NEP activity in genital tissue. This immunohistochemistry kit permits localization of NEP in tissue sections and cultured cells using both light and electron microscopy which may be used for both research and clinical purposes. Such information may be useful for diagnostic and possibly therapeutic purposes in the detection and/or prevention and/or treatment of MED. For each kit the range, sensitivity, precision, reliability, specificity and reproducibility of the assay are established. Intraassay and interassay variation is established at 20%, 50% and 80% points on the standard curves of displacement or activity.

PROBES

Another aspect of the subject invention is the provision of nucleic acid hybridisation or PCR probes which are capable of detecting (especially those that are capable of selectively selecting) polynucleotide sequences, including genomic sequences,

encoding a target coding region or closely related molecules, such as alleles. The specificity of the probe, i.e., whether it is derived from a highly conserved, conserved or non-conserved region or domain, and the stringency of the hybridisation or amplification (high, intermediate or low) will determine whether the probe identifies only naturally occurring target coding sequence, or related sequences. Probes for the detection of related nucleic acid sequences are selected from conserved or highly conserved nucleotide regions of target family members and such probes may be used in a pool of degenerate probes. For the detection of identical nucleic acid sequences, or where maximum specificity is desired, nucleic acid probes are selected from the non-conserved nucleotide regions or unique regions of the target polynucleotides. As used herein, the term "non-conserved nucleotide region" refers to a nucleotide region that is unique to a target coding sequence disclosed herein and does not occur in related family members.

PCR as described in US-A-4683195, US-A-4800195 and US-A-4965188 provides additional uses for oligonucleotides based upon target sequences. Such oligomers are generally chemically synthesized, but they may be generated enzymatically or produced from a recombinant source. Oligomers generally comprise two nucleotide sequences, one with sense orientation (5'->3') and one with antisense (3'<-5') employed under optimised conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantification of closely related DNA or RNA sequences.

The nucleic acid sequence for a target can also be used to generate hybridisation probes as previously described, for mapping the endogenous genomic sequence. The sequence may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. These include *in situ* hybridisation to chromosomal spreads (Verma *et al* (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York City), flow-sorted chromosomal preparations, or artificial chromosome constructions such as YACs, bacterial artificial chromosomes (BACs), bacterial PI constructions or single chromosome cDNA libraries.

In situ hybridisation of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers are invaluable in extending genetic maps. Examples of genetic maps can be found in Science (1995;

270:410f and 1994; 265:1981f). Often the placement of a gene on the chromosome of another mammalian species may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once a disease or syndrome has been crudely localised by genetic linkage to a particular genomic region any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. between normal, carrier or affected individuals.

ORGANISM

The term "organism" in relation to the present invention includes any organism that could comprise the target and/or products obtained therefrom. Examples of organisms may include a fungus, yeast or a plant.

The term "transgenic organism" in relation to the present invention includes any organism that comprises the target and/or products obtained.

TRANSFORMATION OF HOST CELLS/HOST ORGANISMS

As indicated earlier, the host organism can be a prokaryotic or a eukaryotic organism. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook et al (Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press) and Ausubel *et al.*, Current Protocols in Molecular Biology (1995), John Wiley & Sons, Inc.

If a prokaryotic host is used then the nucleotide sequence may need to be suitably modified before transformation - such as by removal of introns.

In another embodiment the transgenic organism can be a yeast. In this regard, yeast have also been widely used as a vehicle for heterologous gene expression. The species *Saccharomyces cerevisiae* has a long history of industrial use, including its use for heterologous gene expression. Expression of heterologous genes in

Saccharomyces cerevisiae has been reviewed by Goodey et al (1987, Yeast Biotechnology, D R Berry et al, eds, pp 401-429, Allen and Unwin, London) and by King et al (1989, Molecular and Cell Biology of Yeasts, E F Walton and G T Yarronton, eds, pp 107-133, Blackie, Glasgow).

For several reasons *Saccharomyces cerevisiae* is well suited for heterologous gene expression. First, it is non-pathogenic to humans and it is incapable of producing certain endotoxins. Second, it has a long history of safe use following centuries of commercial exploitation for various purposes. This has led to wide public acceptability. Third, the extensive commercial use and research devoted to the organism has resulted in a wealth of knowledge about the genetics and physiology as well as large-scale fermentation characteristics of *Saccharomyces cerevisiae*.

A review of the principles of heterologous gene expression in Saccharomyces cerevisiae and secretion of gene products is given by E Hinchcliffe E Kenny (1993, "Yeast as a vehicle for the expression of heterologous genes", Yeasts, Vol 5, Anthony H Rose and J Stuart Harrison, eds, 2nd edition, Academic Press Ltd.).

Several types of yeast vectors are available, including integrative vectors, which require recombination with the host genome for their maintenance, and autonomously replicating plasmid vectors.

In order to prepare the transgenic Saccharomyces, expression constructs are prepared by inserting the nucleotide sequence of the present invention into a construct designed for expression in yeast. Several types of constructs used for heterologous expression have been developed. The constructs contain a promoter active in yeast fused to the nucleotide sequence of the present invention, usually a promoter of yeast origin, such as the GAL1 promoter, is used. Usually a signal sequence of yeast origin, such as the sequence encoding the SUC2 signal peptide, is used. A terminator active in yeast ends the expression system.

For the transformation of yeast several transformation protocols have been developed. For example, a transgenic Saccharomyces according to the present invention can be prepared by following the teachings of Hinnen et al (1978, Proceedings of the National Academy of Sciences of the USA 75, 1929); Beggs, J D (1978, Nature, London, 275, 104); and Ito, H et al (1983, J Bacteriology 153, 163-168).

The transformed yeast cells are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as LEU2, HIS4 and TRP1, and dominant antibiotic resistance markers such as aminoglycoside antibiotic markers, eg G418.

Another host organism is a plant. The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material. Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). Further teachings on plant transformation may be found in EP-A-0449375.

Thus, the present invention also provides a method of transforming a host cell with a nucleotide sequence that is to be the target or is to express the target. Host cells transformed with the nucleotide sequence may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein produced by a recombinant cell may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing coding sequences can be designed with signal sequences which direct secretion of the coding sequences through a particular prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join the coding sequence to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins (Kroll *DJ et al* (1993) DNA Cell Biol 12:441-53).

REFERENCES

Adaikan, P.G. *et al* (1986), Is vasoactive intestinal polypeptide the principal transmitter involved in human penile erection. *J. Auton. Pharmacol.* **135:** 638-640.

Argiolas, A. *et al* (1995), Neuromodulation of penile erection. *Prog Neurobiol.* **47:** 235-255.

Benet, A.E. *et al* (1994), Male erectile dysfunction assessment and treatment options. *Comp. Ther.* **20:** 669-673.

Boolel, M. *et al* (1996). Sildenafil, a novel effective oral therapy for male erectile dysfunction. *Br. J. of Urology* **78**: 257-261.

Carter AJ. *et al* (1998). Effect of the selective phosphodiesterase type 5 inhibitor sildenafil on erectile dysfunction in the anesthetized dog. *J. Urol.* **160**: 242-6.

Chiou, W.F. *et al* (1998). Relaxation of corpus cavernosum and raised intracavernous pressure by berberine in rabbit. *Br. J. Pharmacol.* **125**: 1677-1684.

Christ, G.J. *et al* (1991). Intercellular communication through gap junctions: Potential role in pharmacomechanical coupling and syncytical tissue contraction in vascular smooth muscle isolated from the human corpus cavernosum. *Life Sci.* **49**(24): PL195-200.

Christ, G.J. *et al* (1993). Characterisation of K currents in cultures human corporal smooth muscle cells. *J. Androl.* **14**(5): 319-328.

Christ, G.J. *et al* (1995). Endothelin-1 as a putative modulator of erectile dysfunction: Characterisation of contraction of isolated corporal tissue strips. *J. of Urology* **153:** 1998-2003.

Chuang, A.T. *et al* (1998). Sildenafil, a type-5 cGMP phosphodiesterase inhibitor, specifically amplifies endogenous cGMP-dependent relaxation in rabbit corpus cavernosum smooth muscle *in vitro*. *J. Urology* **160**: 257-261.

Hedlund, H. *et al* (1985), Effects of some peptides on isolated human penile erectile tissue and cavernous artery. *Acta Physiol. Scand.* **124:** 413-419.

Jeremy, J.Y. *et al* (1997). Effects of sildenafil, a type-5 cGMP phosphodiesterase inhibitor, and papaverine on cyclic GMP and cyclic AMP levels in the rabbit corpus cavernosum *in vitro*. *Br. J. Urology* **79**: 958-963.

Kiely, E.A. *et al* (1989), Penile response to intracavernosal vasoactive intestinal polypeptide alone and in combination with other vasoactive agents. *Br J. Urol.* **64:** 191-194.

Lerner, S.E. *et al* (1993). A review of erectile dysfunction: new insights and more questions. *J. Urology* **149:** 1246-1255.

Melman,A. & Gingell, J.C. (1999). The epidemiology and pathophysiology of erectile dysfunction. *J. Urology* **161:** 5-11.

Montague, D. *et al* (1996). Clinical guidelines panel on erectile dysfunction: Summary report on the treatment of organic erectile dysfunction. *J. Urology* **156:** 2007-2011.

Naylor, A.M. (1998). Endogenous neurotransmitters mediating penile erection. *Br. J. Urology* **81:** 424-431.

NIH Consensus Development Panel on Impotence (1993). NIH Consensus Conference *Impotence. J.A.M.A.* **270**: 83.

Omote M. (1999). Pharmacological profiles of sildenafil (VIAGRA) in the treatment of erectile dysfunction: efficacy and drug interaction with nitrate. *Nippon Yakurigaku Zasshi.* **114**:213-8.

Roy, J.B. *et al* (1990), A clinical trial of intracavernous vasoactive intestinal polypeptide to induce penile erection. *J. Urol.* **143:** 302-304.

Steers, W.D. *et al* (1984), Anatomical localisation and some pharmacological effects of vasoactive intestinal polypeptide in human and monkey corpus cavernosum. *J. Urol.* **132:** 1048-1053.

Taub, H.C. *et al* (1993). Relationship between contraction and relaxation in human and rabbit corpus cavernosum. *Urology* **42**: 698-704.

Traish AM, et al (1999). Effects of castration and androgen replacement on erectile function in a rabbit model. *Endocrinology.* **140**: 1861-8.

Turner, AJ. *et al* (1997). Mammalian membrane metallopeptidases: NEP, ECE, KELL and PEX. *FASEB J.* **11**: 355-364.

Wagner, G. *et al* (1987), Intracavernous injection of vasoactive intestinal polypeptide (VIP) does not produce erection in man per se. *World J. Urol.* **5:** 171-177.

Wallis RM. (1999). The pharmacology of sildenafil, a novel and selective inhibitor of phosphodiesterase (PDE) type 5. *Nippon Yakurigaku Zasshi.* **114:** 22P-26P.

Willis, E.A. *et al* (1983), Vasoactive intestinal polypeptide (VIP) as a putative neurotransmitter in penile erection. *Life Sci.* **33:** 383-391.

NEPi – ANIMAL TEST METHODS

Animal models

Anaesthetised Rabbit Methodology

Male New Zealand rabbits (~2.5kg) were pre-medicated with a combination of Medetomidine (Domitor®) 0.5ml/kg *i.m.*, and Ketamine (Vetalar®) 0.25ml/kg *i.m.* whilst maintaining oxygen intake via a face mask. The rabbits were tracheotomised using a Portex™ uncuffed endotracheal tube 3 ID., connected to ventilator and maintained at a ventilation rate of 30-40 breaths per minute, with an approximate tidal volume of 18-20 ml, and a maximum airway pressure of 10 cm H_2O. Anaesthesia was then switched to Isoflurane and ventilation continued with O_2 at 2l/min. The right marginal ear vein was cannulated using a 23G or 24G catheter, and

Lactated Ringer solution perfused at 0.5ml/min. The rabbit was maintained at 3% Isoflurane during invasive surgery, dropping to 2% for maintenance anaesthesia. The left jugular vein was exposed, isolated and then cannulated with a PVC catheter (17G) for the infusion of drugs and compounds. A ventral midline incision was made into the abdominal cavity. The incision was about 5cm in length just above the pubis. The fat and muscle was *bluntly* dissected away to reveal the hypogastric nerve which runs down the body cavity. It was essential to keep close to the side curve of the pubis wall in order to avoid damaging the femoral vein and artery which lie above the pubis. The sciatic and pelvic nerves lie deeper and were located after further dissection on the dorsal side of the rabbit. Once the sciatic nerve is identified, the pelvic nerve was easily located. The term *pelvic nerve* is loosely applied; anatomy books on the subject fail to identify the nerves in sufficient detail. However, stimulation of the nerve causes an increase in vaginal and clitoral blood flow, and innervation of the pelvic region. The pelvic nerve was freed away from surrounding tissue and a *Harvard* bipolar stimulating electrode was placed around the nerve. The nerve was slightly lifted to give some tension, then the electrode was secured in position. Approximately 1ml of light paraffin oil was placed around the nerve and electrode. This acts as a protective lubricant to the nerve and prevents blood contamination of the electrode. The electrode was connected to a *Grass* S88 Stimulator. The pelvic nerve was stimulated using the following parameters:- 5V, pulse width 0.5ms, duration of stimulus 20 seconds with a frequency of 16Hz. Reproducible responses were obtained when the nerve was stimulated every 15-20 minutes. Several stimulations using the above parameters were performed to establish a mean control response. The compound(s) to be tested were infused, via the jugular vein, using a *Harvard* 22 infusion pump allowing a continuous 15 minute stimulation cycle. The skin and connective tissue around the penis was removed to expose the penis. A catheter set (*Insyte-W,* Becton-Dickinson 20 Gauge 1.1 x 48mm) was inserted through the tunica albica into the left *corpus cavernosal* space and the needle removed, leaving a flexible catheter. This catheter was linked via a pressure transducer (Ohmeda 5299-04) to a Gould system to record intracavernosal pressure. Once an intracavernosal pressure was established, the catheter was sealed in place using *Vetbond* (tissue adhesive, 3M). Heart rate was measured via the pulse oxymeter and *Po-ne-mah* data acquisition software system (Ponemah Physiology Platform, Gould Instrument Systems Inc).

Intracavernosal blood flow was recorded either as numbers directly from the Flowmeter using *Po-ne-mah* data acquisition software (Ponemah Physiology Platform, Gould Instrument Systems Inc), or indirectly from Gould chart recorder

trace. Calibration was set at the beginning of the experiment (0-125ml/min/100g tissue). The NEP (Neutral Endopeptidase EC3.4.24.11) inhibitor was made up in saline + 10% 1M NaOH, the phosphodiesterase type 5 (PDE5) inhibitor was made up in saline + 5% 1M HCl. The inhibitors and vehicle controls were infused at a rate of 0.1ml/second. NEP inhibitors and PDE_{cAMP} inhibitors were left for 15 minutes prior to pelvic nerve stimulation.

All data are reported as mean ± s.e.m.. Significant changes were identified using Student's t-tests.

Anaesthetised Dog Methodology

Male beagles, in the range 12-15 kg body weight, were deprived of food overnight. They were anaesthetised with pentobarbitone (30-45mg/kg *i.v.*), and the anaesthesia maintained by a continual infusion of pentobarbitone (60mg/ml) at a rate of 1-1.4ml/h. The left femoral artery was cannulated for the measurement of blood pressure, lead II E.C.G. was recorded and heart-rate derived. A catheter was introduced into the left femoral vein for the administration of compounds. Both ureters were cannulated *via* a mid-line abdominal incision to prevent urine accumulation in the bladder and the bladder was completely emptied. The left internal pudendal artery was carefully dissected free of surrounding tissues to allow placement of a Transonic flow probe for the measurement of arterial blood flow. The cavernosal branches of both pelvic nerves were dissected free and placed into bipolar stimulating electrodes. The skin around the penis was opened and the corpora cavernosa exposed. A 21g needle, attached by flexible catheter to a pressure transducer, was inserted into the corpus (usually the left) for measurement of both *i.c.* pressure and injection of SNP; the system was filled with heparinised saline (15 to 20 U/ml). In the dog the corpora are totally separate which enabled either or both sides to be used if necessary.

The dogs were respired with a Ugo Basile 5025 dog ventilator adjusted to maintain blood gasses in the range pO_2 95-115 mmHg; pCO_2 25-40 mmHg. Expired air was continually monitored by a Datex Normocap 200 to aid respiratory control. Body temperature was maintained within the range 36-38^{0}C using an electric blanket. Parameters were recorded on a Gould TA4000 polygraph and all data acquisition and calculation of derived parameters was carried out on-line using a Po-Ne-Mah system. The cavernosal branches of the pelvic nerves were stimulated with a Grass

S88 stimulator at 10 volts, 2 ms duration for <1 min. At the end of the experiment dogs were killed by an *i.v.* injection of 20ml saturated potassium chloride, whilst still under pentobarbitone anaesthesia. Following a period of equilibration, the pelvic nerves were stimulated at 16Hz in order to assess whether the rise in *i.c.* pressure was rapidly and fully registered by the transducer and changes in blood flow were detected. Control responses were obtained to nerve stimulation at either 1 or 2Hz, On recovery a second stimulation was performed, at double the first frequency. In some dogs a third frequency was used. This stimulation cycle was repeated after 30 min. NEP inhibitors were dissolved in alkaline saline and given as a series of two-tiered infusions starting with a loading infusion and a maintenance infusion for 30 minutes, when the second set of infusions was started. Subsequent infusions were started either at 30 min intervals or when *i.c.* pressure had returned to baseline. All Infusions were given at a rate of 1ml/min. Stimulation cycles were started fifteen minutes into each infusion.

In addition, arterial blood samples were taken from the abdominal aorta, *via* the blood pressure cannula, pre-dose and at 15 and 30minutes into each infusion, for subsequent analysis of unbound compound concentration by Drug Metabolism.

NEPi - TEST RESULTS and DISCUSSION

There are a number of anaesthetised animal models of erection which mimic the physiology of penile erection, i.e. increases in penile blood flow and intracavernosal pressure. The effects of sexual arousal are mimicked by stimulation of pelvic neurones that innervate the penis. This is an accepted mechanism to investigate erectile mechanisms and to assess the potential of potential therapeutic agents for the treatment of MED.

It is well established that selective PDE5 inhibitors such as sildenafil enhance nerve stimulated-increases in intracavernosal pressure (ICP) in animal models and that nerve stimulation mimics the erectile process observed in man (Carter et al., 1998, Traish et al., 1999, Omote 1999, Wallis 1999). This PDE5 inhibitor-induced enhancement of ICP characterises the mechanism of action of PDE5 inhibitors and explains how agents such as sildenafil overcomes any relaxant deficiencies associated with MED or impotence. In agreement with these previous studies, the examples hereinafter have demonstrated that a selective PDE5 inhibitor,

administered intravenously, potentiates nerve-stimulated increases in ICP in the anaesthetised rabbit and dog (Examples 2, 4, 5).

The examples hereinafter demonstrate that inhibition of NEP EC3.4.24.11 with a selective NEP inhibitor dose-dependently potentiates nerve stimulated increases in intracavernosal pressure in the anaesthetised dog (Examples 1, 2 and 3). At the doses used in this study a similar enhancement of the erectile process was observed with a NEPi as was observed with a PDE5 inhibitor (Example 2). Simultaneously recording intracavernosal pressure (ICP) and cavernosal blood flow illustrated that a selective NEP inhibitor enhanced both ICP and cavernosal blood flow (Example 3). These examples underline the potential clinical application of a NEP inhibitor therapy to enhance the erectile process and hence in the treatment of MED.

Examples 4 and 5 demonstrate that concomitant inhibition of NEP EC3.4.24.11 and PDE5 produced a marked enhancement of the ICP, or the erectile process, than was achievable with the same dose of the same PDE5 inhibitor alone. Using the rabbit model of erection, it has been demonstrated that the potentiation of ICP induced by PDE5 inhibition can be further potentiated by co-administration of a NEP EC3.4.24.11 inhibitor (via intravenous administration of a NEPi, 1mg/kg; Example 4). At 1mg/kg (iv) doses of PDE5 inhibitor we observe a maximal potentiation of ICP, the finding that the ICP can be further potentiated beyond this maximal PDE5 inhibitor mediated is highly unexpected. This data illustrates that there are a number of clinical benefits of concomitant administration of a PDE5 inhibitor and a NEP inhibitor over PDE5 inhibitor therapy alone. These include increased efficacy and opportunities to treat MED subgroups that do not respond to PDE5 inhibitor therapy.

It is a particular object of the present invention to provide pharmaceutical compositions comprising a NEPi and a PDEi for use in the treatment of MED wherein the specific combination provides synergistic benefits.

In addition the onset of action of PDE5 inhibitors i.e. the time taken to reach maximal effect is greatly reduced in the presence of a NEP EC 3.4.24.11 inhibitor (Example 5). Clinically this represents a quicker onset time.

In addition, co-administration of a NEPi and a PDE5i allows the onset of action of PDE5i to be reduced. Hence there is a quickening of the time between agent administration and clinical endpoint.

Inhibitors of NEP EC3.4.24.11 and PDE5 or combinations of the two, have no significant effect on un-stimulated ICP i.e. they do not directly induce an increase in ICP in the absence of sexual drive/arousal. This is highly advantageous as the only other marketed therapy for MED which requires sexual stimulation to work is sildenafil thus the present invention provides a viable alternative oral therapy to sildenafil and all other PDE5 alone based drugs.

NEPi - ANIMAL MODEL EXAMPLES

Compounds used:

NEPi: a preferred compound of general formula I as defined herein before.

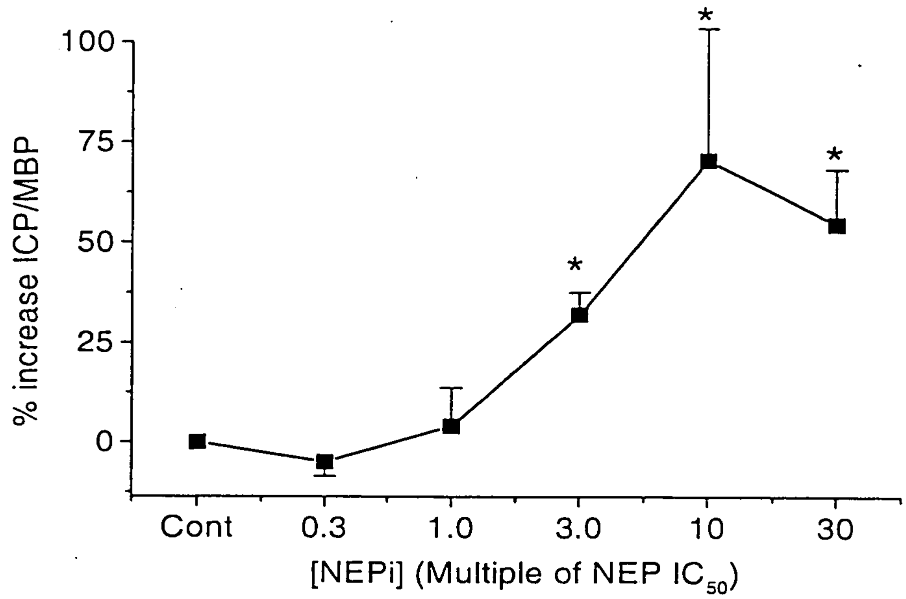
PDE5i: 3-ethyl-5-{5-[4-ethylpiperzino)-2-propoxyphenyl}-2-(-pyridylmethyl)-6,7-dihydro-2H-pyrazolo[4,3-*d*]pyrimidin-7-one.

Example 1. Inhibition of NEP EC3.4.24.11 dose-dependently potentiates nerve stimulated increases in intracavernosal pressure in anaesthetised dog model of erection.

Submaximal increases in intracavernosal pressure (ICP) induced by nerve-stimulation were significantly increased in the presence of increasing doses of a selective NEP EC3.4.24.11 inhibitor (iv infusion to steady state concentrations). The maximal potentiation (circa 70%) was observed at around 10 times the IC50 value obtained against native NEP. Data is expressed as the percentage (%) increase, compared to control stimulated increases, in ICP divided by mean blood pressure (MBP) and multiplied by 100. Values are expressed as mean $\pm$ s.e.mean. * $P<0.01$, Students t-test unpaired compared with control increases.

There were no major effects of NEP inhibition on basal/un-stimulated intracavernosal pressure.

Inhibition of NEP dose-dependantly potentiates nerve-stimulated erections in an anaesthetised dog model of erection

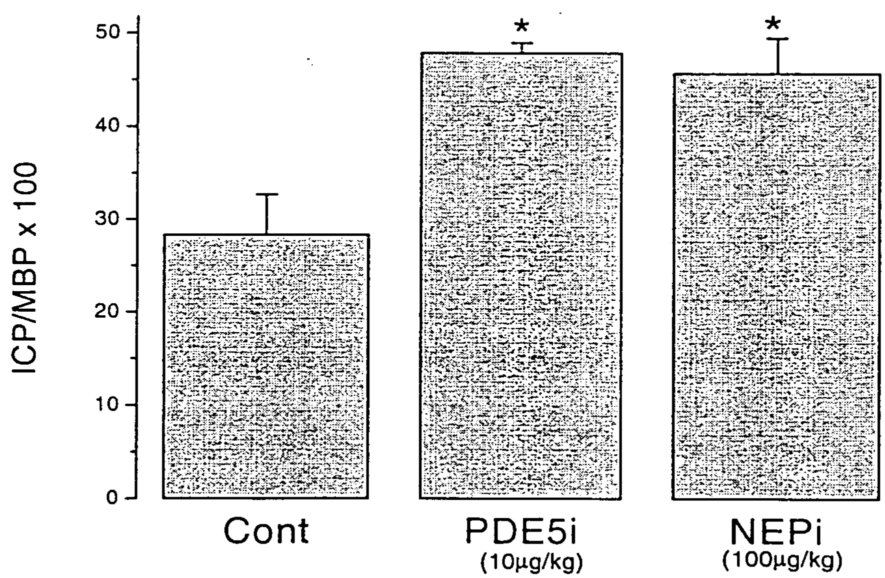


Example 2. Inhibition of PDE5 or NEP EC3.4.24.11 potentiates nerve stimulated increases in intracavernosal pressure in anaesthetised dog model of erection.

Submaximal increases in intracavernosal pressure (ICP) induced by nerve-stimulation were significantly increased in the presence of a selective PDE5 inhibitor (10μg/kg; iv bolus) NEP EC3.4.24.11 inhibitor (100μg/kg; iv bolus). The maximal potentiation for both agents was circa 65% at the doses used. Data is expressed as ICP divided by mean blood pressure (MBP) and multiplied by 100. Values are expressed as mean ± s.e.mean. * P<0.01, Students t-test unpaired compared with control increases.

There were no major effects of NEP or PDE5 inhibition on basal/un-stimulated intracavernosal pressure.

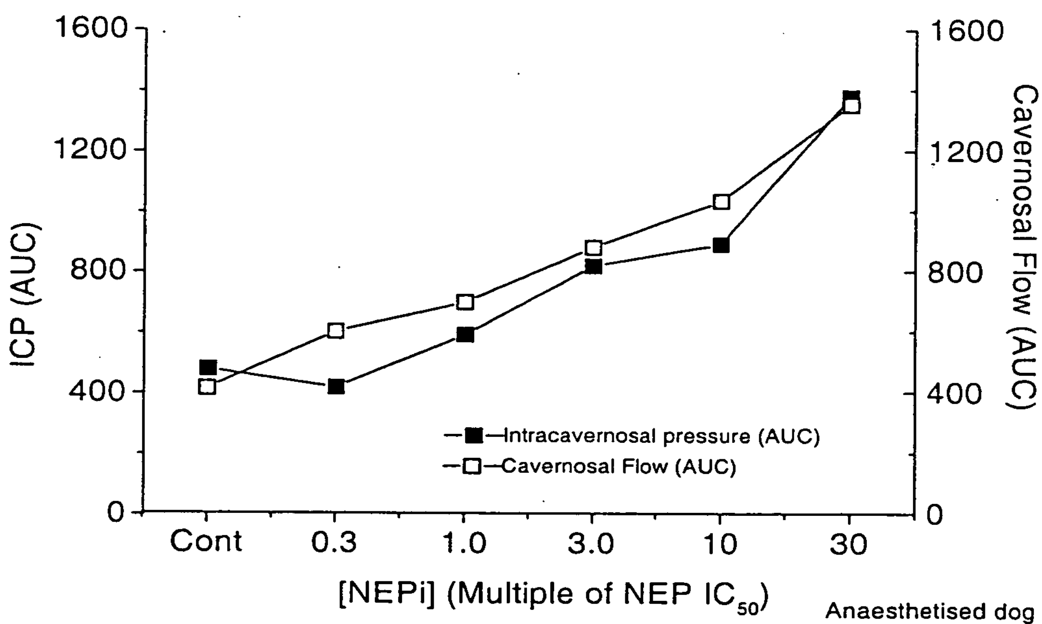
Inhibition of NEP or PDE5 potentiates nerve-stimulated erections in an anaesthetised dog model of erection



Example 3. NEP inhibition dose-dependently potentiates nerve stimulated increases in intracavernosal pressure and cavernosal blood flow in anaesthetised dog model of erection.

Submaximal increases in intracavernosal pressure (ICP) and increases in cavernosal blood flow induced by nerve-stimulation were increased in the presence of increasing doses of a selective NEP EC3.4.24.11 inhibitor (iv infusion to steady state concentrations). ICP was increased circa 188% whereas flow was increased circa 228%. Data for ICP and flow, both expressed as area under the curve (AUC), were recorded simultaneously from a single animal.

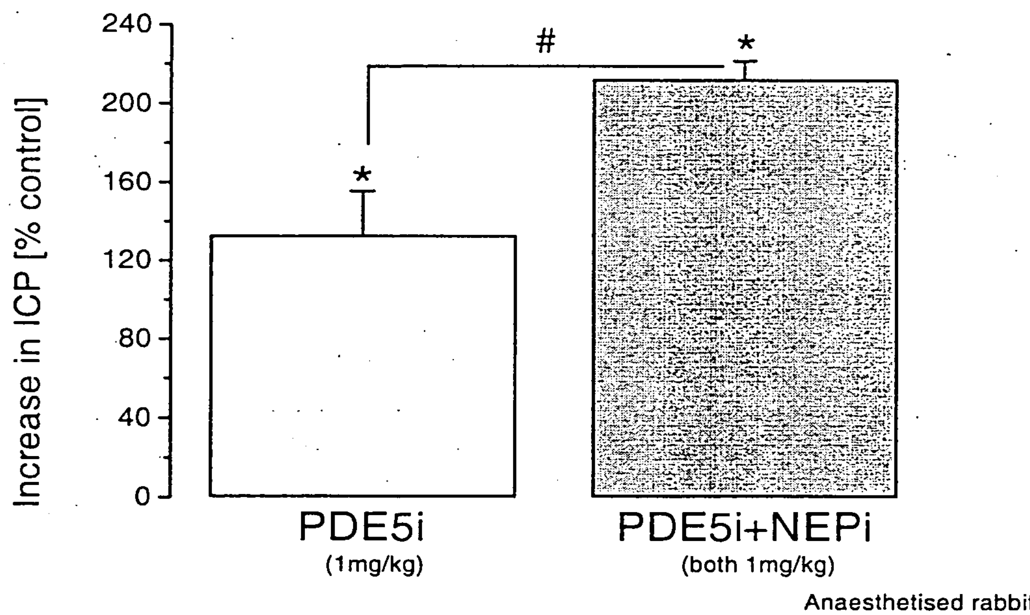
Inhibition of NEP dose-dependantly potentiates nerve-stimulated increases in intracavernosal pressure and cavernosal blood flow



Example 4. NEP inhibition significantly increases the efficacy of PDE5 inhibitor to enhance penile erection in an anaesthetised rabbit model of erection.

Intravenous administration of a selective PDE5 inhibitor (1 mg/kg) significantly enhanced nerve-stimulated increases in ICP by 133±22% compared to control increases. Once the PDE5i-mediated increase was sustained, co-administration of a selective NEP EC3.4.24.11 inhibitor further enhanced nerve-stimulated increases in ICP. This represents a NEP inhibition-induced potentiated of 79% (P<0.01, paired t-test) compared to increases observed with a PDE5 inhibitor. Data is expressed as percentage increase in ICP over control increases. Values are expressed as mean ± s.e.mean. * P<0.01, Students t-test unpaired compared with control increases.
There were no effects of PDE5 inhibition or combined PDE5/NEP inhibition on basal/un-stimulated intracavernosal pressure.

Concommitant inhibition of NEP and PDE5 significantly potentiates the PDE5-mediated enhancement of nerve-stimulated erection



Example 5. NEP inhibition potentiates the erectile effects of PDE5 inhibitors and speeds up the onset of action of PDE5 inhibitors in the anaesthetised rabbit model of erection.

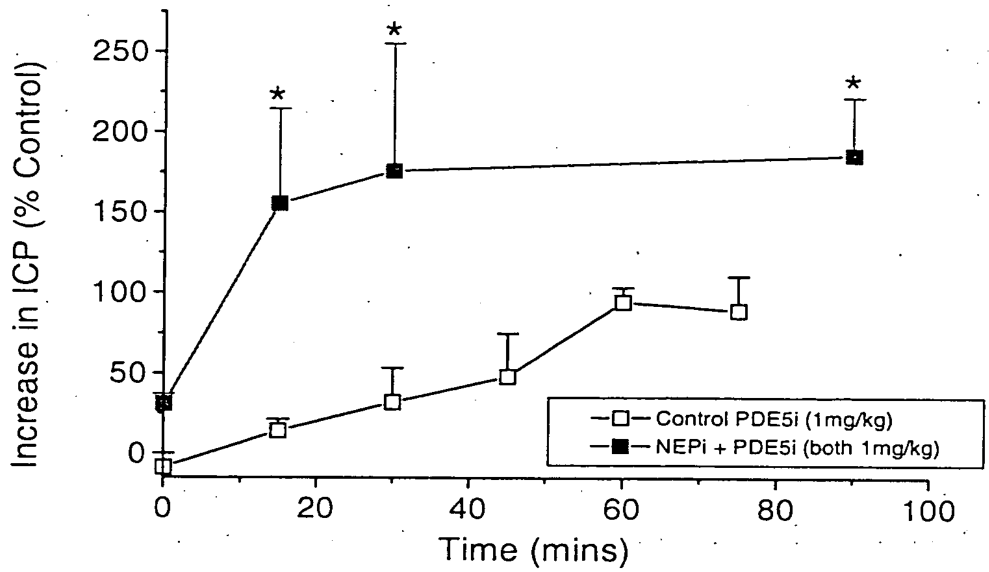
Concomitant inhibition of NEP EC3.4.24.11 and PDE5 significantly potentiates the PDE5 inhibitor-mediated enhancement of nerve-stimulated increases in intracavernosal pressure (ICP). Submaximal increases in ICP are significantly enhanced (circa 90% compared to control increases) in the presence of a selective PDE5 inhibitor (1mg/kg; iv bolus). When the same dose of the PDE5 inhibitor is given in the presence of a NEP inhibitor (1mg/kg; iv bolus) a further enhancement of ICP is observed (circa 187% compared to control increases). This represents a NEP inhibitor mediated enhance of PDE5 inhibitor mediated effects of around 100%.

In addition to the increased enhancement of ICP observed on concomitant application of a NEPi and a PDE5i, the time taken for a PDE5 inhibitor to exert it's maximal effect (i.e. onset of action) is reduced in the presence of a NEP inhibitor (22.5 min in the presence compared to 67.5 min in the absence of a NEP inhibitor).

There were no major effects of NEP inhibition or combined PDE5/NEP inhibition on basal/un-stimulated intracavernosal pressure.

Data is expressed as percentage increase in ICP over control increases. Values are expressed as mean $\pm$ s.e.mean. * $P<0.01$, Students t-test unpaired compared with PDE5 inhibitor mediated increases.

Concommitant inhibition of NEP and PDE5 significantly potentiates the PDE5i-mediated enhancement of nerve-stimulated erection



Anaesthetised rabbit

NEP ENZYME ASSAY

THE PREPARATION AND ASSAY OF SOLUBLE (NEP) NEUTRAL ENDOPEPTIDASE FROM CANINE, RAT, RABBIT AND HUMAN KIDNEY CORTEX.

Soluble NEP is obtained from the kidney cortex and activity is assayed by measuring the rate of cleavage of the NEP substrate Abz-D-Arg-Arg-Leu-EDDnp to generate its fluorescent product, Abz-D-Arg-Arg.

EXPERIMENTAL PROCEDURE:-

1. MATERIALS

All water is double de ionised.

1.1 Tissues

Human Kidney	IIAM (Pennsylvania. U.S.A.)
Rat Kidney	
Rabbit Kidney	

Canine Kidney

1.2 Homogenisation medium

100mM Mannitol and 20mM Tris @ pH 7.1

2.42g Tris (Fisher T/P630/60) is diluted in 1 litre of water and the pH adjusted to 7.1 using 6M HCl at room temperature. To this 18.22g Mannitol (Sigma M-9546) is added.

1.3 Tris buffer (NEP buffer).

50ml of 50mM Tris pH 7.4 (Sigma T2663) is diluted in 950ml of water.

1.4 Substrate (Abz-D-Arg-Arg-Leu-EDDnp)

Made to order from SNPE, and is stored as a powder at –20°C. A 2mM stock is made by gently re-suspending the substrate in Tris buffer, this should not be vortexed or sonicated. 600µl aliquots of the 2mM stock are stored at –20 for up to one month. (Medeiros, M.A.S., Franca, M.S.F. et al., (1997), Brazilian Journal of Medical and Biological Research, 30, 1157-1162).

1.5 Total product

Samples corresponding to 100% substrate to product conversion are included on the plate to enable the % substrate turnover to be determined. The total product is generated by incubating 1ml of 2mM substrate with 20µl of enzyme stock for 24 hours at 37°C.

1.6 Stop solution.

A 300µM stock of Phosphoramidon (Sigma R7385) is made up in NEP buffer and stored in 50µl aliquots at -20.

1.7 Dimethyl sulphoxide (DMSO).

1.8 Magnesium Chloride -$MgCl_2.6H_2O$ (Fisher M0600/53).

1.9 Black 96 well flat bottom assay plates (Costar 3915).

1.10 Topseal A (Packard 6005185).

1.11 Centrifuge tubes

2. SPECIFIC EQUIPTMENT

2.1 Sorvall RC-5B centrifuge (SS34 GSA rotor, pre-cooled to 4°C).

2.2 Braun miniprimer mixer.

2.3 Beckman CS-6R centrifuge.

2.4 Fluostar galaxy.

2.5 Wesbart 1589 shaking incubator.

3. METHODS

3.1 TISSUE PREPARATION

3.2 Dog, rat, rabbit, and human NEP is obtained from the kidney cortex using a method adapted from Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Frozen kidneys are allowed to thaw at room temperature and the cortex is dissected away from the medulla.

3.4 The cortex is finely chopped and homogenised in approximately 10 volumes of homogenisation buffer (1.2) using a Braun miniprimer (2.2).

3.5 Magnesium chloride (1.8) (20.3mg/gm tissue) is added to the homogenate and stirred in an ice-water bath for 15 minutes.

3.6 The homogenate is centrifuged at 1,500g (3,820rpm) for 12 minutes in a Beckman centrifuge (2.3) before removing the supernatant to a fresh centrifuge tube and discarding the pellet.

3.7 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes in a Sovall centrifuge (2.1) and the supernatant is discarded.

3.8 The pale pink layer on the top of the remaining pellet is removed and re-suspended in homogenisation buffer containing magnesium chloride (9mg MgCl in 5ml buffer per 1g tissue).

3.9 The suspension is centrifuged at 2,200g (4,630rpm) for 12 minutes in a Beckman centrifuge (2.3) before discarding the pellet.

3.10 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes using the Sorvall centrifuge (2.1) and the supernatant is discarded.

3.11 The final pellet is resuspended in homogenisation buffer containing magnesium chloride (0.9mg MgCl in 0.5ml buffer per 1g tissue). A homogenous suspension is obtained using a Braun miniprimer (2.2). This is then frozen down in 100µl aliquots to be assayed for NEP activity.

4.0 DETERMINATION OF NEP ACTIVITY

The activity of the previously aliquoted NEP is measured by its ability to cleave the NEP specific peptide substrate.

4.1 A 4% DMSO/NEP buffer solution is made (4mls DMSO in 96mls NEP buffer).

4.2 Substrate, total product, enzyme, and Phosphoramidon stocks are left on ice to thaw.

4.3 50µl of 4% DMSO/NEP buffer solution is added to each well.

4.4 The 2mM substrate stock is diluted 1:40 to make a 50µM solution. 100µl of 50µM substrate is added to each well (final concentration 25µM).

4.5 50µl of a range of enzyme dilutions is added to initiate the reaction (usually 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 are used). 50µl of NEP buffer is added to blank wells.

4.6 The 2mM total product is diluted 1:80 to make a 25µM solution. 200µl of 25µM product is added to the first four wells of a new plate.

4.7 Plates are incubated at 37oC in a shaking incubator for 60 minutes.

4.8 The 300µM Phosphoramidon stock is diluted 1:100 to 300nM. The reaction is stopped by the addition of 100µl 300nM Phosphoramidon and incubated at 37^{0}C in a shaking incubator for 20 minutes before being read on the Fluostar (ex320/em420).

5. NEP INHIBITION ASSAYS

5.1 Substrate, total product, enzyme and Phoshoramidon stocks are left on ice to thaw.

5.2 Compound stocks are made up in 100% DMSO and diluted 1:25 in NEP buffer to give a 4% DMSO solution. All further dilutions are carried out in a 4% DMSO solution (4mls DMSO in 96mls NEP buffer).

5.3 50µl of compound in duplicate is added to the 96 well plate and 50µl of 4% DMSO/NEP buffer is added to control and blank wells.

5.4 The 2mM substrate stock is diluted 1:40 in NEP buffer to make a 50µM solution (275µl 2mM substrate to 10.73ml buffer is enough for 1 plate).

5.5 The enzyme stock diluted in NEP buffer (determined from activity checks).

5.6 The 2mM total product stock is diluted 1:80 in NEP buffer to make a 25µM solution. 200µl is added to the first four wells of a separate plate.

5.7 The 300µM Phosphoramidon stock is diluted 1:1000 to make a 300nM stock (1.1µl Phosphoramidon to 10.99ml NEP buffer.

5.8 To each well in the 96 well plate the following is added:

Table Reagents to be added to 96 well plate.

	Compound/ DMSO	Tris Buffer	Substrate	NEP enzyme	Total product
Samples	2µl compound	50µl	100µl	50µl	None
Controls	2µl DMSO	50µl	100µl	50µl	None
Blanks	2µl DMSO	100µl	100µl	None	None
Totals	2µl DMSO	None	None	None	200µl

5.9 The reaction is initiated by the addition of the NEP enzyme before incubating at 37°C for 1 hour in a shaking incubator.

5.10 The reaction is stopped with 100µl 300nM Phosphoramidon and incubated at 37°C for 20 minutes in a shaking incubator before being read on the Fluostar (ex320/em420).

6. CALCULATIONS

The activity of the NEP enzyme is determined in the presence and absence of compound and expressed as a percentage.

% Control activity (turnover of enzyme):

$$\frac{\text{Mean FU of controls} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

% Activity with inhibitor:

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

Activity expressed as % of control:

$$\frac{\text{\% Activity with inhibitor}}{\text{\% Control activity}} \times 100$$

A sigmoidal dose-response curve is fitted to the % activities (% of control) vs compound concentration and IC50 values calculated using LabStats fit-curve in Excel.

ACE ASSAY

THE PREPARATION AND ASSAY OF SOLUBLE ANGIOTENSIN CONVERTING ENZYME (ACE), FROM PORCINE AND HUMAN KIDNEY CORTEX.

Soluble ACE activity is obtained from the kidney cortex and assayed by measuring the rate of cleavage of the ACE substrate Abz-Gly-p-nitro-Phe-Pro-OH to generate its fluorescent product, Abz-Gly.

1. MATERIALS

All water is double de ionised.

1.1 Human Kidney IIAM (Pennsylvania. U.S.A.) or UK Human

Tissue Bank (UK HTB)

1.2 Porcine kidney ACE Sigma (A2580)

1.3 Homogenisation buffer-1

100mM Mannitol and 20mM Tris @ pH 7.1

2.42g Tris (Fisher T/P630/60) is diluted in 1 litre of water and the pH adjusted to 7.1 using 6M HCl at room temperature. To this 18.22g Mannitol (Sigma M-9546) is added.

1.4 Homogenisation buffer-2

100mM Mannitol, 20mM Tris @ pH7.1 and 10mM $MgCl_2.6H_2O$ (Fisher M0600/53)

To 500ml of the homogenisation buffer 1 (1.4) 1.017g of $MgCl_2$ is added.

1.5 Tris buffer (ACE buffer).

50mM Tris and 300mM NaCl @ pH 7.4

50ml of 50mM Tris pH 7.4 (Sigma T2663) and 17.52g NaCl (Fisher S/3160/60) are made up to 1000ml in water.

1.6 Substrate (Abz-D-Gly-p-nitro-Phe-Pro-OH) (Bachem M-1100)

ACE substrate is stored as a powder at –20°C. A 2mM stock is made by gently re-suspending the substrate in ACE buffer, this must not be vortexed or sonicated. 400µl aliquots of the 2mM stock are stored at –20°C for up to one month.

1.7 Total product

Samples corresponding to 100% substrate to product conversion are included on the plate to enable the % substrate turnover to be determined (see calculations). The total product is generated by incubating 1ml of 2mM substrate with 20µl of enzyme stock for 24 hours at 37°C.

1.8 Stop solution.

0.5M EDTA (Promega CAS[6081/92/6]) is diluted 1:250 in ACE buffer to make a 2mM solution.

1.9 Dimethyl sulphoxide (DMSO).

1.10 Magnesium Chloride -$MgCl_2.6H_2O$ (Fisher M0600/53).

1.11 Black 96 well flat bottom assay plates (Costar 3915 or Packard).

1.12 Topseal A (Packard 6005185).

1.13 Centrifuge tubes

2. SPECIFIC EQUIPTMENT

2.1 Sorvall RC-5B centrifuge (SS34 GSA rotor, pre-cooled to 4°C).

2.2 Braun miniprimer mixer.

2.3 Beckman CS-6R centrifuge.

2.4 BMG Fluostar Galaxy.

2.5 Wesbart 1589 shaking incubator.

3. METHODS

3.1 TISSUE PREPARATION

3.3 Human ACE is obtained from the kidney cortex using a method adapted from Booth, A.G. & Kenny, A.J. (1974) *Biochem. J.* 142, 575-581.

3.3 Frozen kidneys are allowed to thaw at room temperature and the cortex is dissected away from the medulla.

3.4 The cortex is finely chopped and homogenised in approximately 10 volumes of homogenisation buffer-1 (1.4) using a Braun miniprimer (2.2).

3.5 Magnesium chloride (1.11) (20.3mg/gm tissue) is added to the homogenate and stirred in an ice-water bath for 15 minutes.

3.6 The homogenate is centrifuged at 1,500g (3,820rpm) for 12 minutes in a Beckman centrifuge (2.3) before removing the supernatant to a fresh centrifuge tube and discarding the pellet.

3.7 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes in a Sovall centrifuge (2.1) and the supernatant is discarded.

3.8 The pale pink layer on the top of the remaining pellet is removed and re-suspended in homogenisation buffer-2 (1.5) (5ml buffer per 1g tissue).

3.9 The suspension is centrifuged at 2,200g (4,630rpm) for 12 minutes in a Beckman centrifuge before discarding the pellet.

3.10 The supernatant is centrifuged at 15,000g (12,100rpm) for 12 minutes using the Sorvall centrifuge and the supernatant is discarded.

3.11 The final pellet is resuspended in homogenisation buffer-2 (0.5ml buffer per 1g tissue). A homogenous suspension is obtained using a Braun miniprimer. This is then frozen down in 100µl aliquots to be assayed for NEP activity.

4.0 DETERMINATION OF ACE ACTIVITY

The activity of the previously aliquoted ACE is measured by its ability to cleave the ACE specific peptide substrate.

Porcine ACE (1.2) is defrosted and resuspended in ACE buffer (1.6) at 0.004U/µl, this is frozen down in 50µl aliquots.

4.1 A 4% DMSO/ACE buffer solution is made (4mls DMSO in 96mls ACE buffer).

4.2 Substrate (1.7), total product (1.8) and enzyme (1.1, 1.2, 1.3), are left on ice to thaw.

4.3 50µl of 4% DMSO/ACE buffer solution is added to each well.

4.4 The 2mM substrate stock is diluted 1:100 to make a 20µM solution. 100µl of 20µM substrate is added to each well (final concentration in the assay 10µM).

4.5 50µl of a range of enzyme dilutions is added to initiate the reaction (usually 1:100, 1:200, 1:400, 1:800, 1:1600, and 1:3200 are used). 50µl of ACE buffer is added to blank wells.

4.6 The 2mM total product is diluted 1:200 to make 10µM solution. 200µl 10µM product is added to the first four wells of a new plate.

4.7 Plates are incubated at 37°C in a shaking incubator for 60 minutes.

4.8 The enzyme reaction is stopped by the addition of 100µl 2mM EDTA in ACE buffer and incubated at 37°C in a shaking incubator for 20 minutes before being read on the BMG Fluostar Galaxy (ex320/em420).

5. ACE INHIBITION ASSAYS

5.1 Substrate, total product, and enzyme stocks are left on ice to thaw.

5.2 Compound stocks are made up in 100% DMSO and diluted 1:25 in ACE buffer to give a 4% DMSO solution. All further dilutions are carried out in a 4% DMSO/ACE buffer solution (4mls DMSO in 96mls ACE buffer).

5.3 50µl of compound, in duplicate, is added to the 96 well plate and 50µl of 4% DMSO/ACE buffer is added to control and blank wells.

5.4 Steps 5.2 and 5.3 can be carried out either by hand or using the Packard multiprobe robots

5.5 The 2mM substrate stock is diluted 1:100 in ACE buffer to make a 20µM solution (10µM final concentration in the assay) (110µl of 2mM substrate added to 10.89ml buffer is enough for 1 plate).

5.6 The enzyme stock is diluted in ACE buffer, as determined from activity checks (4.0).

5.7 The 2mM total product stock is diluted 1:200 in ACE buffer to make a 10µM solution. 200µl is added to the first four wells of a separate plate.

5.8 The 0.5mM EDTA stock is diluted 1:250 to make a 2mM stock (44µl EDTA to 10.96ml ACE buffer).

5.9 To each well of the 96 well plate the following reagents are added:

Table 1: Reagents added to 96 well plate.

	Compound/ DMSO	Tris Buffer	Substrate	ACE enzyme	Total product
Samples	2µl compound	50µl	100µl	50µl	None
Controls	2µl DMSO	50µl	100µl	50µl	None
Blanks	2µl DMSO	100µl	100µl	None	None
Totals	2µl DMSO	None	None	None	200µl

5.10 50µl of the highest concentration of each compound used in the assay is added in duplicate to the same 96 well plate as the totals (5.7). 150µl of ACE buffer is added to determine any compound fluorescence.

5.11 The reaction is initiated by the addition of the ACE enzyme before incubating at 37°C for 1 hour in a shaking incubator.

5.12 The reaction is stopped by the addition of 100µl 2mM EDTA and incubated at 37°C for 20 minutes in a shaking incubator, before being read on the BMG Fluostar Galaxy (ex320/em420).

6. CALCULATIONS

The activity of the ACE enzyme is determined in the presence and absence of compound and expressed as a percentage.

FU = Fluorescence units

(i) % Control activity (turnover of enzyme):

$$\frac{\text{Mean FU of controls} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

(ii) % Activity with inhibitor:

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of totals} - \text{Mean FU of blanks}} \times 100$$

(iii) Activity expressed as % of control:

$$\frac{\text{\% Activity with inhibitor}}{\text{\% Control activity}} \times 100$$

OR

$$\frac{\text{Mean FU of compound} - \text{Mean FU of blanks}}{\text{Mean FU of controls} - \text{Mean FU of blanks}} \times 100$$

(iv) % Inhibition = 100 - % control

(v) For fluorescent compounds the mean FU of blanks containing compound (5.10) is deducted from the mean FU of compound values used to calculate the % Activity.

A sigmoidal dose-response curve is fitted to the % activities (% of control) vs compound concentration and IC_{50} values calculated using LabStats fit-curve in Excel.

PDE5 inhibitor – TEST METHODS

Phosphodiesterase (PDE) inhibitory activity

Preferred PDE compounds suitable for use in accordance with the present invention are potent and selective cGMP PDE5 inhibitors. *In vitro* PDE inhibitory activities against cyclic guanosine 3',5'-monophosphate (cGMP) and cyclic adenosine 3',5'-monophosphate (cAMP) phosphodiesterases can be determined by measurement of their IC_{50} values (the concentration of compound required for 50% inhibition of enzyme activity).

The required PDE enzymes can be isolated from a variety of sources, including human corpus cavernosum, human and rabbit platelets, human cardiac ventricle, human skeletal muscle and bovine retina, essentially by the method of W.J. Thompson and M.M. Appleman (Biochem., 1971, 10, 311). In particular, the cGMP-specific PDE (PDE5) and the cGMP-inhibited cAMP PDE (PDE3) can be obtained from human corpus cavernosum tissue, human platelets or rabbit platelets; the cGMP-stimulated PDE (PDE2) was obtained from human corpus cavernosum; the calcium/calmodulin (Ca/CAM)-dependent PDE (PDE1) from human cardiac ventricle; the cAMP-specific PDE (PDE4) from human skeletal muscle; and the photoreceptor PDE (PDE6) from bovine retina. Phosphodiesterases 7-11 can be generated from full length human recombinant clones transfected into SF9 cells.

Assays can be performed either using a modification of the "batch" method of W.J. Thompson et al. (Biochem., 1979, 18, 5228) or using a scintillation proximity assay for the direct detection of AMP/GMP using a modification of the protocol described by Amersham plc under product code TRKQ7090/7100. In summary, the effect of PDE inhibitors was investigated by assaying a fixed amount of enzyme in the presence of varying inhibitor concentrations and low substrate, (cGMP or cAMP in a 3:1 ratio unlabelled to [^{3}H]-labeled at a conc ~1/3 K_m) such that $IC_{50} \cong K_i$. The final assay volume was made up to 100μl with assay buffer [20 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, 1 mg/ml bovine serum albumin]. Reactions were initiated with enzyme, incubated for 30-60 min at 30°C to give <30% substrate turnover and terminated with 50 μl yttrium silicate SPA beads (containing 3 mM of the respective unlabelled cyclic nucleotide for PDEs 9 and 11). Plates were re-sealed and shaken for 20 min, after which the beads were allowed to settle for 30 min in the dark and then counted on a TopCount plate reader (Packard, Meriden, CT) Radioactivity units were converted to

% activity of an uninhibited control (100%), plotted against inhibitor concentration and inhibitor IC_{50} values obtained using the 'Fit Curve' Microsoft Excel extension.

Especially preferred for use herein herein are compounds which have an IC_{50} value of less than about 10, more preferably less than about 5, and most preferably less than about 2nM for the PDE5 enzyme in combination with selectivity of greater than 10-fold, more preferably greater than 50-fold, more preferably greater than 100-fold and especially greater than 200-fold selectivity for the PDE5 enzyme versus the PDE6 enzyme.

Functional activity

This can be assessed in vitro by determining the capacity of a compound of the invention to enhance sodium nitroprusside-induced relaxation of pre-contracted rabbit corpus cavernosum tissue strips, as described by S.A. Ballard et al. (Brit. J. Pharmacol., 1996, 118 (suppl.), abstract 153P).

In vivo activity

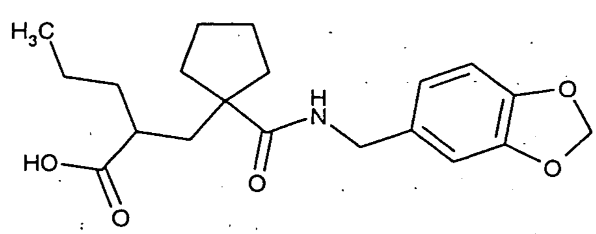
Compounds were screened in anaesthetised dogs to determine their capacity, after i.v. administration, to enhance the pressure rises in the corpora cavernosa of the penis induced by intracavernosal injection of sodium nitroprusside, using a method based on that described by Trigo-Rocha et al. (Neurourol. and Urodyn., 1994, 13, 71).

CHEMICAL COMPOUND EXAMPLES and Preparations

NEPi Examples

Example 1

2-({1-[(1,3-Benzodioxol-5-ylamino)carbonyl]cyclopentyl}methyl)pentanoic acid

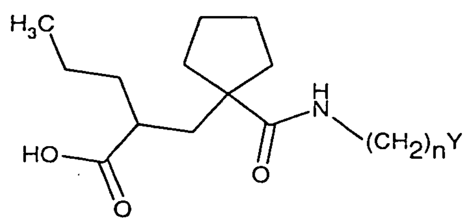


Trifluoroacetic acid (5ml) was added to a solution of the *tert*-butyl ester from preparation 34 (130mg, 0.31mmol) in dichloromethane (5ml), and the solution stirred

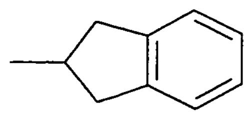
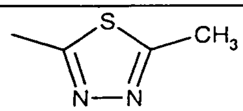
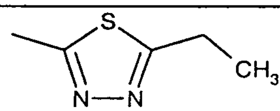
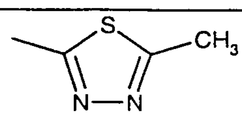
at room temperature for 4 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with toluene and dichloromethane to afford the title compound as a clear oil, 112 mg, ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.83 (t, 3H), 1.22-1.40 (m, 3H), 1.50-1.72 (m, 8H), 1.95 (m, 1H), 2.10 (m, 2H), 2.19 (m, 1H), 4.30 (m, 2H), 5.93 (s, 2H), 5.99 (bs, 1H), 6.74 (m, 3H); LRMS: m/z 380 (MH^-).

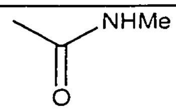
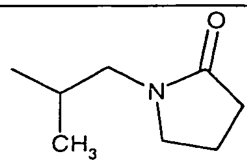
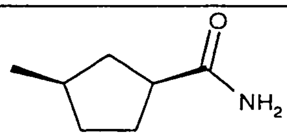
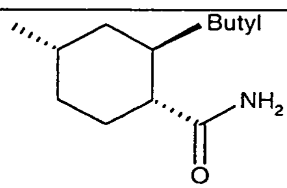
Examples 2 to 9

COMPOUNDS OF FORMULA IC, I.E. COMPOUNDS OF GENERAL FORMULA I WHERE R^1 IS PROPYL, WHERE PREPARED FROM THE CORRESPONDING *TERT*-BUTYL ESTER, FOLLOWING A SIMILAR PROCEDURE TO THAT DESCRIBED IN EXAMPLE 1.



(Ic)

Ex	n	R	Yield	Data
2[1]	0		78	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (s, 3H), 1.17-2.04 (m, 14H), 2.27-2.38 (m, 1H), 2.64-2.80 (m, 2H), 3.20-3.31 (m, 2H), 4.60-4.72 (m, 1H), 5.97 (d, 1H), 7.03-7.18 (m, 4H). LRMS : m/z 343.8 (M^+).
3[2,3]	0		81	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.30-1.42 (m, 4H), 1.59-1.81 (m, 7H), 2.18 (m, 1H), 2.30 (m, 1H), 2.42 (m, 1H), 2.55 (m, 1H), 2.61 (s, 3H). LRMS : m/z 324 (MH^-). Mp 184-186°C Anal. Found: C, 55.50; H, 7.22; N, 12.61. $C_{15}H_{23}N_3O_3S$ requires C, 55.36; H, 7.14; N, 12.91%.
4[3]	0		86	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.92 (t, 3H), 1.35 (t, 3H), 1.25-1.80 (m, 11H), 2.20-2.50 (m, 4H), 2.95 (q, 2H), 12.10 (bs, 1H). LRMS : m/z 339.8 (MH^+) Anal. Found: C, 56.46; H, 7.46; N, 12.36. $C_{16}H_{25}N_3O_3S$ requires C, 56.62; H, 7.44; N, 12.37%.
5[2]	1		81	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H), 1.20-1.70 (m, 11H), 1.90-2.20 (m, 3H), 2.25 (m, 1H), 2.70 (s, 3H), 4.75 (m, 2H), 7.10 (bs, 1H). LRMS : m/z 340.6 (MH^+)

Ex	n	R	Yield	Data
6[2]	2		45	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.25-1.40 (m, 3H), 1.41-1.70 (m, 8H), 1.92 (m, 1H), 2.00-2.18 (m, 2H), 2.38 (m, 1H), 2.42 (t, 2H), 2.80 (d, 3H), 3.40-3.60 (m, 2H), 6.50 (bs, 1H), 6.74 (bs, 1H). LRMS : m/z 313.2 (MH^+)
7	0		93	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.19 (d, 3H), 1.21-1.69 (m, 11H), 1.89-2.10 (m, 5H), 2.30 (m, 1H), 2.41 (m, 2H), 2.95 (m, 1H), 3.35 (m, 1H), 3.63 (m, 2H), 4.20 (m, 1H), 6.58-6.70 (m, 1H). LRMS : m/z 353.1 (MH^+)
8	0		99	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 3H), 1.20-1.39 (m, 3H), 1.41-2.10 (m, 1H), 2.80 (m, 1H), 4.35 (m, 17H), 5.81 (d, 1H), 6.30 (bs, 0.5H), 6.43 (bs, 0.5H), 7.40 (bd, 0.5H), 7.61 (bd, 0.5H). LRMS : m/z 339.8 (MH^+)
9	0			^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (m, 6H), 1.08-2.08 (m, 29H), 4.29 (m, 1H), 5.95 (d, 1H), 6.43 (s, 1H), 7.80 (d, 1H). LRMS : m/z 409.5 (MH^+)

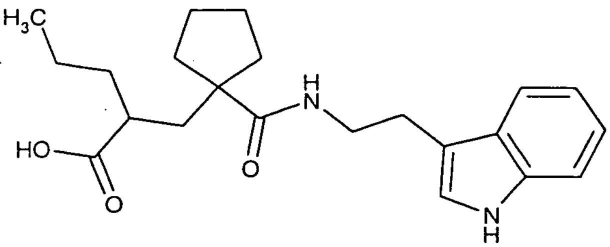
1 = additionally purified by column chromatography on silica gel using ethyl acetate:pentane as eluant.

2 = additionally purified by column chromatography on silica gel using dichloromethane:methanol as eluant.

3 = recrystallised from ether

Example 10

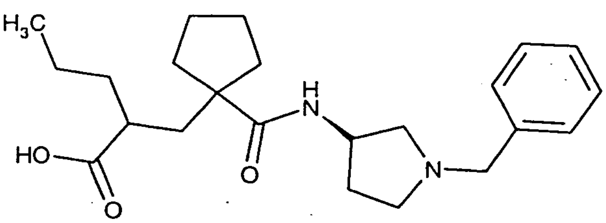
2-{[1-({[2-(1*H*-Indol-3-yl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



Trifluoroacetic acid (2.61ml, 33.9mmol) was added to a solution of the *tert*-butyl ester from preparation 44 (482mg, 1.13mmol) and anisole (1.23ml, 11.3mmol) in dichloromethane (4ml), and the reaction stirred at room temperature for 4 hours. The mixture was washed with water, then brine, dried ($MgSO_4$), concentrated under reduced pressure and the residue azeotroped with toluene. The residual brown oil was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant, and re-columned using an elution gradient of ethyl acetate:pentane (30:70 to 50:50) to afford the title compound as a clear foam, 136mg, 32%; ^{1}HNMR ($CDCl_3$, 400MHz) δ: 0.82 (s, 3H), 1.16-1.77 (m, 12H), 1.78-2.03 (m, 2H), 2.36 (m, 1H), 2.97 (m, 2H), 3.61 (m, 2H), 5.83 (m, 1H), 7.04 (s, 1H), 7.09-7.23 (m, 2H), 7.39 (d, 1H), 7.61 (d, 1H), 8.15 (m, 1H); LRMS : m/z 371.8 (MH^+.

Example 11

2-{[1-({[(3*S*)-1-Benzylpyrrolidinyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid

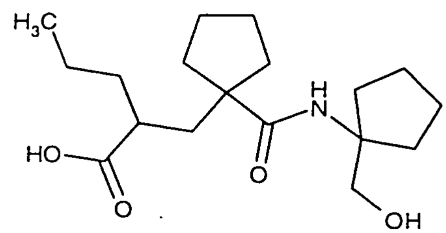


A solution of the *tert*-butyl ester from preparation 45 (70mg, 0.16mmol) in trifluoroacetic acid (1ml) and dichloromethane (1ml) was stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure and the residue azeotroped with dichloromethane. The residue was partitioned between water (1ml) and ethyl acetate (5ml), and the pH of the aqueous layer adjusted to 6 using sodium bicarbonate solution. The layers were separated, the organic phase dried (Na_2SO_4), evaporated under reduced pressure and the residue azeotroped with dichloromethane, to give the title compound as a beige foam, 45mg, 73%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-2.95 (m, 19H), 3.52 (m, 1H), 3.75 (m, 1H), 3.95

(m, 1H), 4.25 (m, 1H), 4.45 (m, 1H), 6.96 (bs, 1H), 7.39 (m, 5H); LRMS : m/z 387 (MH^+); Anal. Found: C, 61.11; H, 7.69; N, 6.00. $C_{23}H_{34}N_2O_3$;CH_2Cl_2 requires C, 61.14; H, 7.70; N, 5.94%.

Example 12

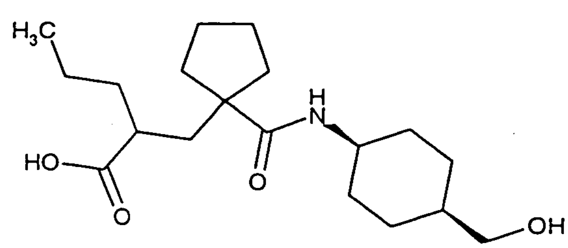
2-{[1-({[1-(Hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



A solution of the *tert*-butyl ester from preparation 33 (38mg, 0.1mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml) was stirred at room temperature for 2 hours. The reaction was concentrated under reduced pressure and the residue azeotroped with toluene and then dichloromethane to give a colourless gum. This was suspended in a solution of potassium carbonate (50mg, 0.3mmol) in methanol, and the mixture stirred for 2 hours at room temperature. The methanol was removed under reduced pressure, the residual aqueous mixture diluted with water (20ml), and acidifed to pH 2 using 2N hydrochloric acid. This solution was extracted with ethyl acetate (2x20ml), and the combined organic solutions dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil, 32mg, 97%; ^1H NMR ($CDCl_3$, 400MHz) δ:: 0.88 (t, 3H), 1.20-1.40 (m, 3H), 1.41-1.90 (m, 17H), 2.01-2.20 (m, 2H), 2.40 (m, 1H), 3.71 (dd, 2H), 5.80 (bs, 1H); LRMS : m/z 326.1 (MH^+)

Example 13

Cis-2-{[1-({[4-(Hydroxymethyl)cyclohexyl]amino}carbonyl)cyclopentyl]methyl} pentanoic acid

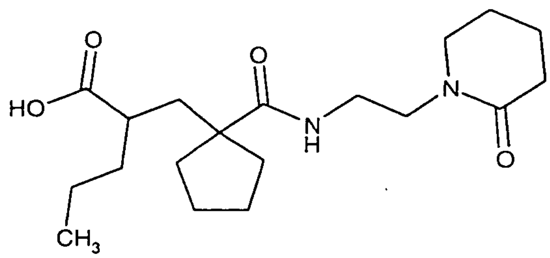


The title compound was obtained as a colourless gum in 68%, from the *tert*-butyl ester from preparation 43, following the procedure described in example 12, except

the product was additionally purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as the eluant; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.87 (t, 3H), 1.21-1.40 (m, 6H), 1.52-1.70 (m, 15H), 1.92-2.11 (m, 3H), 2.39 (m, 1H), 3.55 (d, 2H), 4.01 (m, 1H), 5.90 (m, 1H); LRMS : m/z 340.3 (MH^+).

Example 14

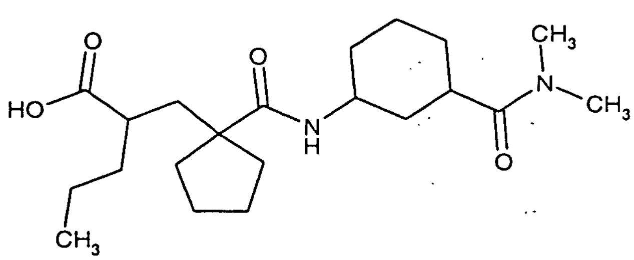
2-{[1-({[2-(2-Oxo-1-piperidinyl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



Hydrogen chloride gas was bubbled through an ice-cold solution of the *tert*-butyl ester from preparation 47 (43mg, 0.105mmol) in dichloromethane (10ml), for 20 minutes. The solution was then stirred at room temperature for 3 hours. The mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give a glass-like solid. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound, 6mg; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.81 (t, 3H), 1.20-1.36 (m, 4H), 1.41-1.69 (m, 7H), 1.79 (m, 4H), 1.90-2.10 (m, 3H), 2.30 (m, 1H), 2.38 (t, 2H), 3.30-3.60 (m, 6H), 7.00 (bs, 1H); LRMS : m/z 351 $(M-H)^-$.

Example 15

2-({1-[({3-[(Dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]cyclopentyl}methyl) pentanoic acid

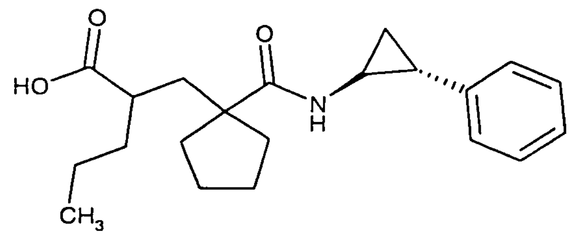


The title compound was obtained as a solid in 85% yield, from the *tert*-butyl ester from preparation 42, following a similar method to that described in example 14, except that dichloromethane:methanol:acetic acid (95:3:2) was used as the

chromatographic eluant; ^{1}H NMR ($CDCl_3$, 400MHz) d: 0.89 (t, 3H), 1.09-1.76 (m, 12H), 1.80-2.17 (m, 10H), 2.37 (m, 1H), 2.68 (m, 1H), 2.95 (s, 3H), 3.04 (s, 3H), 3.83 (m, 1H), 6.06 (m, 1H); LRMS : m/z 381 (MH^+); Anal. Found: C, 63.31; H, 9.17; N, 6.53. $C_{21}H_{36}N_2O_4$;H_2O requires C, 63.29; H, 9.61; N, 7.03%.

Example 16

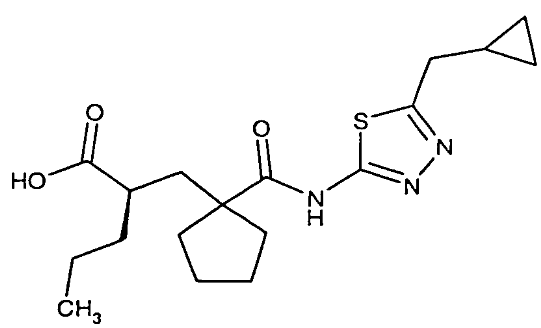
2-{[1-({[(1*R*,2*R*)-2-Phenylcyclopropyl]amino}carbonyl)cyclopentyl]methyl}pentanoic acid



The title compound was obtained quantitatively as an orange gum from the *tert*-butyl ester from preparation 46, following a similar procedure to that described in example 14; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.12-2.14 (m, 17H), 2.38 (m, 1H), 2.87 (m, 1H), 6.10 (s, 1H), 7.13 (m, 3H), 7.25 (m, 2H); LRMS : m/z 344.3 (MH^+).

Example 17

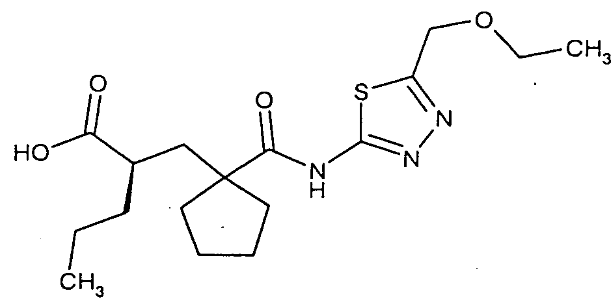
(2*R*)-2-{[1-({[5-(Cyclopropylmethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl] methyl}pentanoic acid



A solution of the *tert*-butyl ester from preparation 50 (63mg, 0.15mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml), was stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant , to give the title compound as a white foam, 46mg, 83%; ^{1}H NMR (CD_3OD, 400MHz) δ: 0.38 (m, 2H), 0.62 (m, 2H), 0.82 (t, 3H), 1.12 (m, 1H), 1.26 (m, 2H), 1.38 (m, 1H), 1.52 (m, 1H), 1.78-1.78 (m, 6H), 1.90 (m, 1H), 2.23 (m, 4H), 2.92 (d, 2H); LRMS : m/z 366.0 (MH^+); $[\alpha]_D$ = -7.75° (c = 0.08, methanol).

Example 18

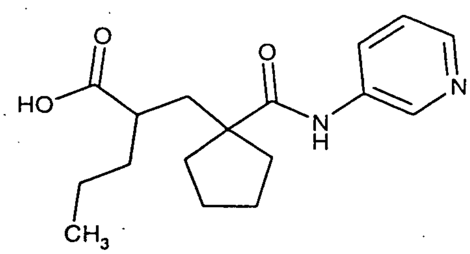
(2*R*)-2-{[1-({[5-(Ethoxymethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid



The title compound was obtained as a white foam in 62% yield, from the *tert*-butyl ester from preparation 51, following a similar procedure to that described in example 17; ^{1}H NMR (CD_3OD, 400MHz) δ: 0.82 (t, 3H), 1.21-1.40 (m, 7H), 1.50 (m, 1H), 1.60-1.77 (m, 7H), 1.88 (m, 1H), 2.23 (m, 4H), 3.62 (q, 2H); $[\alpha]_D$ = -6.08° (c = 0.25, methanol).

Example 19

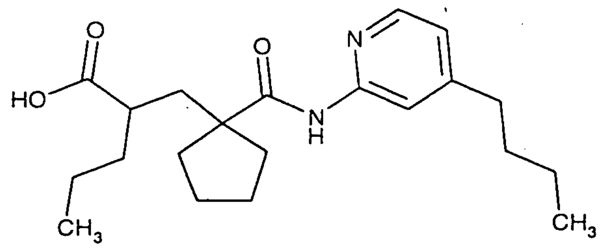
2-({1-[(3-Pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 52 (130mg, 0.33mmol) and 10% palladium on charcoal (20mg) in 95% aqueous ethanol (3ml) was hydrogenated at 15psi and room temperature for 2 hours. The reaction was filtered through Arbocel®, washing through with ethanol, and the filtrate evaporated under reduced pressure. The residual gum was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to afford the title compound, 103mg, 83%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 2H), 1.44 (m, 1H), 1.58-1.82 (m, 8H), 2.19 (m, 1H), 2.39 (m, 2H), 2.52 (m, 1H), 6.88 (m, 1H), 7.67 (m, 1H), 7.82 (d, 1H), 8.38 (d, 1H), 9.78 (s, 1H); LRMS : m/z 305 (MH^+).

Example 20

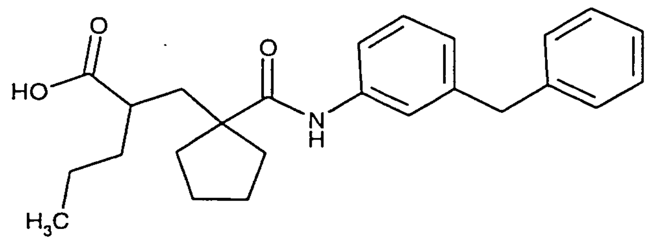
2-[(1-{[(4-Butyl-2-pyridinyl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid



The title compound was obtained in 92% yield from the benzyl ester from preparation 55, following a similar procedure to that described in example 19; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.90 (m, 6H), 1.28-1.50 (m, 5H), 1.58-1.81 (m, 10H), 2.20 (m, 1H), 2.40 (m, 2H), 2.58 (m, 3H), 6.70 (d, 1H), 7.68 (d, 1H), 8.22 (s, 1H), 9.90 (bs, 1H).

Example 21

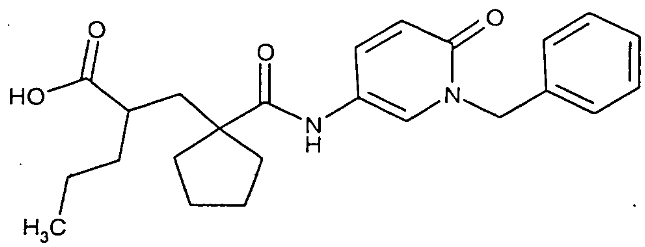
2-({1-[(3-Benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 53 (1.3mg, 2.47mmol) and 5% palladium on charcoal (130mg) in water (10ml) and ethanol (40ml) was hydrogenated at 30 psi and room temperature for 2 hours. The reaction mixture was fiiltered through Arbocel®, the filtrate concentrated under reduced pressure, and the residue triturated with dichloromethane. The residual gum was triturated with ether, then hexane, and dried at 50°C, to give the title compound as a solid, 0.79g, 81%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.95 (t, 3H), 1.24-1.51 (m, 3H), 1.58-1.80 (m, 7H), 1.88 (dd, 1H), 2.15 (m, 2H), 2.24 (m, 1H), 2.48 (m, 1H), 4.00 (s, 2H), 6.98 (d, 1H), 7.24 (m, 6H), 7.40 (m, 3H); Anal. Found: C, 75.48; H, 7.76; N, 3.59. $C_{25}H_{31}NO_3$;$0.25H_2O$ requires C, 75.44; H, 7.98; N, 3.51%.

Example 22

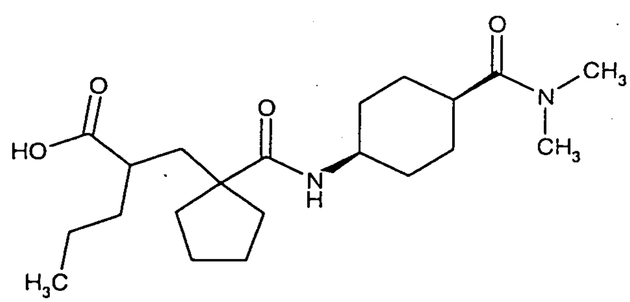
2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]-pentanoic acid.



The title compound was obtained as a white foam in 51% yield from the benzyl ester from preparation 56, following a similar procedure to that described in example 21, except, the product was purified by column chromatography on silica gel, using ethyl acetate as eluant; [1]H NMR ($CDCl_3$, 300MHz) δ: 0.96 (t, 3H), 1.28-1.80 (m, 12H), 2.01 (m, 1H), 2.30-2.52 (m, 2H), 5.02 (dd, 2H), 6.60 (d, 1H), 7.27 (m, 5H), 7.70 (s, 1H), 8.34 (s, 1H); Anal. Found: C, 69.52; H, 7.41; N, 6.51. $C_{24}H_{30}N_2O_4$;$0.25H_2O$ requires C, 69.45; H, 7.41; N, 6.75.

Example 23

Cis-2-({1-[({4-[(Dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoic acid

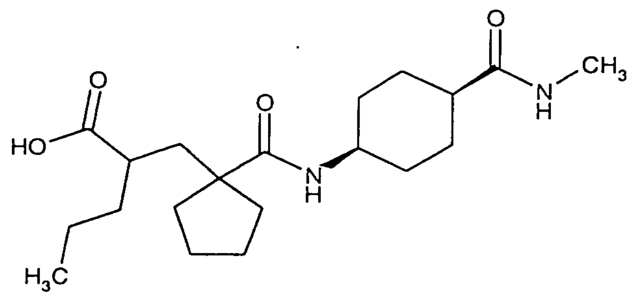


A mixture of the benzyl ester from preparation 58 (150mg, 0.33mmol) and 10% palladium on charcoal (20mg) in water (0.3ml) and ethanol (3.5ml) was hydrogenated at 15 psi and room temperature for 3 days. The reaction mixture was filtered through Arbocel®, and the filtrate concentrated under reduced pressure. The residual gum was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to afford the title compound, 85mg, 65%; [1]H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.29-1.96 (m, 18H), 2.01-2.23 (m, 4H), 2.37 (m, 1H), 2.62 (m, 1H), 2.96 (s, 3H), 3.03 (s, 3H), 3.96 (m, 1H), 5.98 (m, 1H);

LRMS : m/z 381.8 (MH^+); Anal. Found: C, 63.81; H, 9.58; N, 6.99. $C_{21}H_{36}N_2O_4$;$0.2CH_2Cl_2$ requires C, 64.06; H, 9.23; N, 7.05%.

Example 24

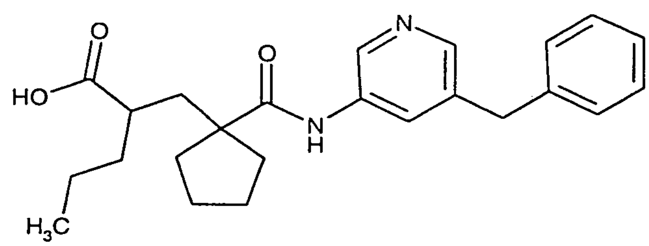
Cis-2-({1-[({4-[(Methylamino)carbonyl]cyclohexyl}amino)carbonyl]cyclopentyl}-methyl)pentanoic acid



The title compound was obtained as a white solid in 34% yield from the benzyl ester from preparation 59, following the procedure described in example 23; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.90 (t, 3H), 1.26-2.02 (m, 20H), 2.19 (m, 3H), 2.39 (m, 1H), 2.82 (d, 3H), 4.00 (m, 1H), 5.69 (m, 1H), 6.00 (d, 1H); LRMS : m/z 365 ($M-H^-$).

Example 25

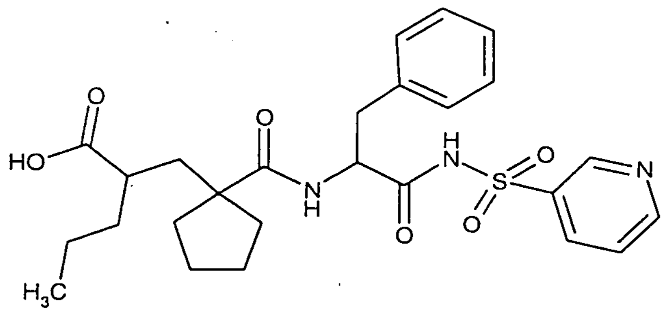
2-[(1-{[(5-Benzyl-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-pentanoic acid.



A mixture of the benzyl ester from preparation 54 (850mg, 1.76mmol) and 5% palladium on charcoal (100mg) in 20% aqueous ethanol (30ml) was hydrogenated at 30 psi and room temperature for 2 hours. The mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure, and the residue azeotroped with dichloromethane to give the title compound as a foam, 0.63g; ^1H NMR ($CDCl_3$, 300MHz) δ: 0.92 (t, 3H), 1.30-1.83 (m, 11H), 2.07 (m, 1H), 2.42 (m, 3H), 3.82 (s, 2H), 7.15-7.38 (5H), 7.80 (s, 1H), 8.48 (s, 1H), 8.59 (s, 1H), 8.62 (s, 1H); Anal. Found: C, 72.29; H, 7.70; N, 6.90. $C_{24}H_{30}N_2O_3$;$0.25H_2O$ requires C, 72.24; H, 7.70; N, 7.02%.

Example 26

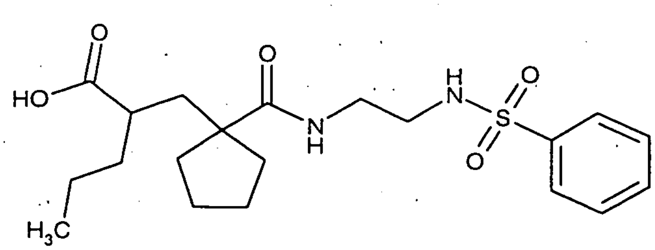
2-({1-[({1-Benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethyl}amino)-carbonyl]cyclopentyl}methyl)pentanoic acid.



A mixture of the benzyl ester from preparation 57 (918mg, 1.52mmol) and 10% palladium on charcoal (90mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 4 ½ hours. Tlc analysis showed starting material remaining, so additional catalyst (70mg) was added, and the mixture hydrogenated for a further 18 hours. Tlc analysis, again showed starting material remaining, so further catalyst (70mg) was added, and hydrogenation continued for an additional 6 hours. The reaction mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure and the residue azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:acetic acid:ethanol (99:1:0 to 79.1:0.9:20) to afford the title compound as a white foam, 271mg, 35%; ^{1}H NMR ($DMSOd_6$, 300MHz) δ: 0.75 (m, 3H), 0.96-1.42 (m, 11H), 1.61-1.99 (m, 4H), 2.75-3.02 (m, 2H), 4.45 (m, 1H), 7.20 (m, 6H), 7.62 (m, 1H), 8.24 (m, 1H), 8.83 (s, 1H), 9.01 (s, 1H), 11.98 (bs, 1H), 12.70 (bs, 1H); IR (KBr disc) 1185, 1195 (m), 1455, 1515, 1640, 1704, 2870, 2930, 2960 (s).

Example 27

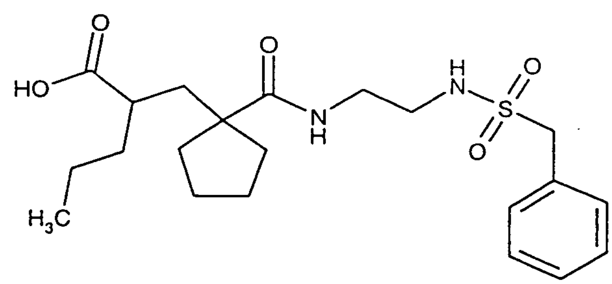
2-({1-[({2-[(Phenylsulfonyl)amino]ethyl}amino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the amine from preparation 61 (235mg, 0.72mmol), benzenesulphonyl chloride (127mg, 0.72mmol) and triethylamine (150µl, 1.08mmol) in dichloromethane (6ml) was stirred at room temperature for 2 days. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) as eluant to give a clear oil. This was then dissolved in trifluoroacetic acid (3ml) and dichloromethane (3ml) and the solution stirred at room temperature for 6 hours. The mixture was concentrated under reduced pressure and the residue azeotroped twice with toluene. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) to afford the title compound as a clear oil, 204mg, 69%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.22-1.43 (m, 4H), 1.43-2.18 (m, 10H), 2.36 (m, 1H), 3.11 (m, 2H), 3.20-3.31 (m, 1H), 3.42-3.53 (m, 1H), 6.13-6.24 (m, 1H), 7.42-7.59 (m, 3H), 7.84 (m, 2H); LRMS : m/z 411.8 (MH^+); Anal. Found: C, 57.26; H, 7.40; N, 6.61. $C_{20}H_{30}N_2O_5S$ requires C, 57.18; H, 7.22; N, 6.62%.

Example 28

2-({1-[({2-[(Benzylsulfonyl)amino]ethyl}amino)carbonyl]cyclopentyl}methyl)pentanoic acid

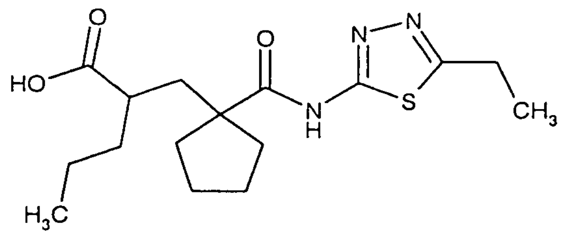


The title compound was obtained as a clear oil in 97% yield, from the amine from preparation 61, following the procedure described in example 27, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.87 (t, 3H), 1.19-1.72 (m, 11H), 1.80-1.96 (m, 1H), 2.00-2.16 (m, 2H), 2.27-2.38 (m, 1H), 2.92-3.21 (m, 3H), 3.23-3.39 (m, 1H), 4.25 (s, 2H), 5.80-6.06 (m, 1H), 6.38 (m, 1H), 7.29-7.43 (m, 5H); LRMS : m/z 425.8 (MH^+).

Example 29
(-)-2-[(1-{[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid

and

Example 30
(+)-2-[(1-{[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid



The acid from Example 4 (824mg) was further purified by HPLC using an AD column and using hexane:*iso*-propanol:trifluoroacetic acid (85:15:0.2) as eluant to give the title compound of example 29 as a white foam, 400mg, 99.5% ee, ^1H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.36 (m, 6H), 1.50-1.80 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.30 (bs, 1H), LRMS : m/z 338 (MH^-), $[\alpha]_D$ = -9.0° (c = 0.1, methanol), and the title compound of example 30 as a white foam, 386mg, 99% ee, ^1H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 6H), 1.50-1.79 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.27 (bs, 1H); LRMS: m/z 338 (MH^-); and $[\alpha]_D$ = +3.8° (c = 0.1, methanol)

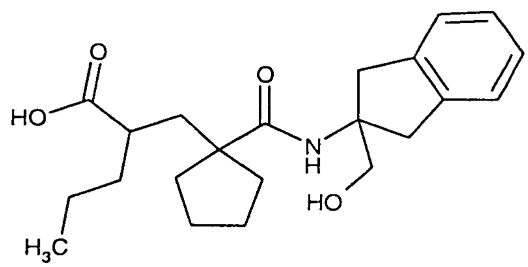
Example 31

(+)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid

and

Example 32

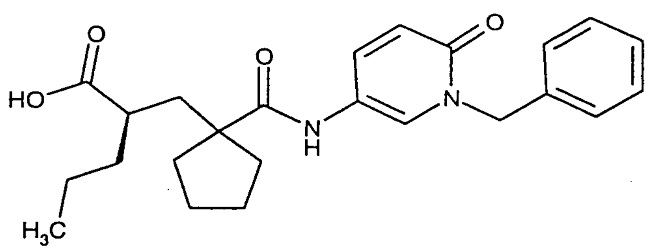
(-)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1H-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid



2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid (WO 9110644) was further purified by HPLC using an AD column and hexane:isopropanol:trifluoroacetic acid (90:10:0.1) as eluant, to give the title compound of example 31, 99% ee, $[\alpha]_D = +10.4°$ (c = 0.067, ethanol) and the title compound of example 32, 99% ee, $[\alpha]_D = -10.9°$ (c = 0.046, ethanol).

Example 33

(2*R*)-2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]-pentanoic acid.

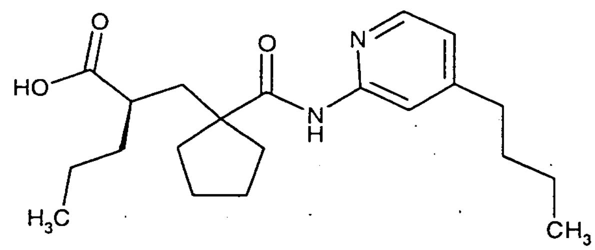


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (191mg, 1.0mmol), 1-hydroxybenzotriazole hydrate (135mg, 01.0mmol), N-methylmorpholine (165μl, 1.5mmol) and finally the amine from preparation 28 (150mg, 0.69mmol) were added to a solution of the acid from preparation 2 (284mg, 1.0mmol) in N,N-dimethylformamide (8ml), and the reaction stirred at 90°C for 18 hours. The cooled solution was diluted with ethyl acetate (90ml), washed with water (4x50ml), and brine (50ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using ethyl acetate:pentane

(30:70) to give a yellow oil, 191mg. This intermediate was dissolved in dichloromethane (3ml) and trifluoroacetic acid (3ml) and the solution stirred at room temperature for 5 hours. The mixture was concentrated under reduced pressure and the residue purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as eluant to give the title compound as a foam, 77mg, ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (t, 3H), 1.20-1.76 (m, 12H), 1.93-2.02 (m, 1H), 2.20-2.46 (m, 3H), 4.95 (d, 1H), 5.04 (d, 1H), 6.61 (d, 1H), 7.21 (m, 1H), 7.50 (s, 1H), 8.23 (s, 1H); LRMS : m/z 411.6 (MH)+; $[\alpha]_D$ = -3.8° (c = 0.052, ethanol).

Example 34

(2*R*)-2-[(1-{[(4-Butyl-2-pyridinyl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid

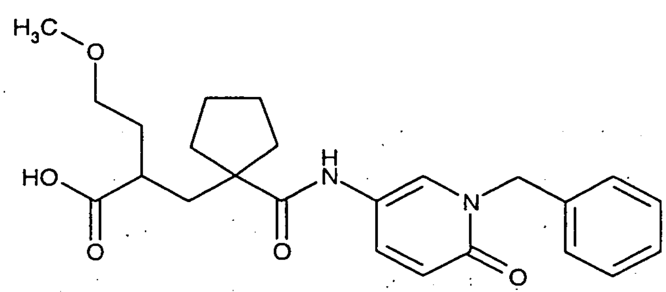


The title compound was obtained in 43% yield from the acid from preparation 2 and the amine from preparation 30, following a similar procedure to that described in example 33, ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80-1.00 (m, 6H), 1.22-1.84 (m, 18H), 2.03-2.56 (m, 3H), 2.77 (m, 1H), 7.14 (d, 1H), 8.08 (d, 1H), 8.23 (s, 1H), 11.71 (brs, 1H).

LRMS : m/z 361.7 $(MH)^+$, $[\alpha]_D$ = -1.4° (c = 0.14, ethanol).

Example 35

2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-4-methoxybutanoic acid



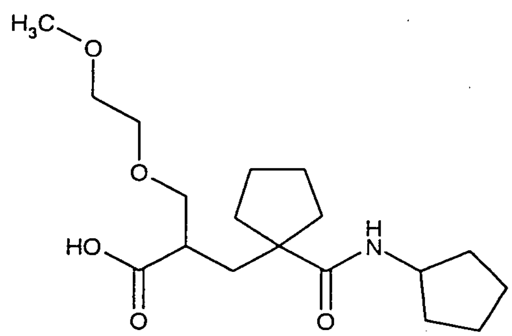
A mixture of the benzyl ester from preparation 62 (850mg, 1.64mmol), and 5% palladium on charcoal (250mg) in 40% aqueous ethanol (21ml), was hydrogenated at 30 psi and room temperature for 30 minutes. The reaction mixture was filtered

through Hyflo®, and the filtrate evaporated under reduced pressure. The residual foam was purified by column chromatography on silica gel using dichloromethane:methanol (97:3) as eluant to give the title compound as a white foam, 550mg, 79%; ^{1}H NMR (DMSO-d_6, 300MHz) δ: 1.24-2.17 (m, 12H), 2.18-2.31 (m, 1H), 3.07 (s, 3H), 3.21 (t, 2H), 5.08 (s, 2H), 6.63 (d, 1H), 7.23-7.41 (m, 5H), 7.72 (d, 1H), 8.24 (s, 1H).

Anal. Found: C, 67.46; H, 7.18; N, 6.24. $C_{24}H_{30}N_2O_5$ requires C, 67.58; H, 7.09; N, 6.57%.

Example 36

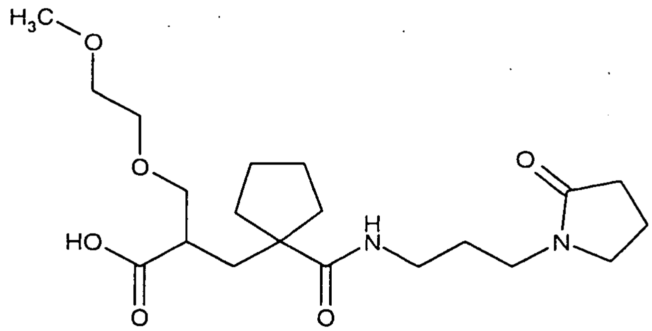
3-{1-[(Cyclopentylamino)carbonyl]cyclopentyl}-2-[(2-methoxyethoxy)methyl]propanoic acid



A solution of the *tert*-butyl ester from preparation 64 (320mg, 0.80mmol) in trifluoroacetic acid (2ml) and dichloromethane (2ml) was stirred at room temperature for 8 hours. The mixture was concentrated under reduced pressure and the residue azeotroped twice with toluene. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) to give the title compound as a clear oil, 171mg, 62%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.29-1.40 (m, 2H), 1.42-1.69 (m, 10H), 1.75 (dd, 1H), 1.87-2.03 (m, 5H), 2.64 (m, 1H), 3.34 (s, 3H), 3.43-3.52 (m, 3H), 3.57 (m, 2H), 3.61 (m, 1H), 4.08-4.20 (m, 1H), 5.89 (d, 1H); LRMS : m/z 340 (MH^-).

Example 37

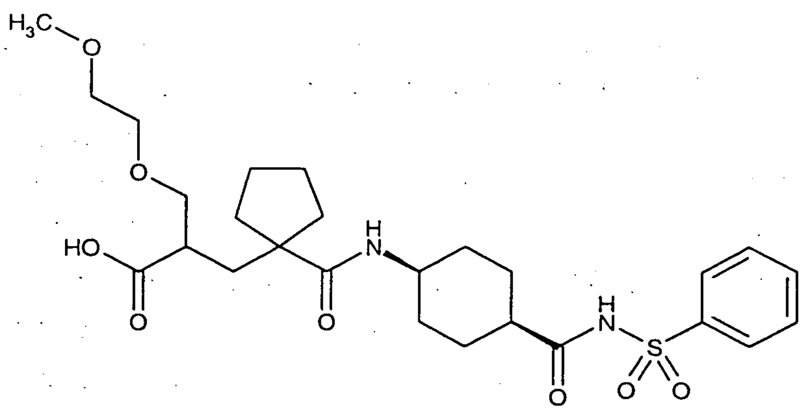
3-(2-Methoxyethoxy)-2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)-cyclopentyl]methyl}propanoic acid



The title compound was obtained as a clear oil in 57% yield from the *tert*-butyl ester of preparation 65, following the procedure described in example 36, ^1H NMR ($CDCl_3$, 300MHz) δ: 1.56-1.78 (m, 8H), 1.94-2.17 (m, 6H), 2.44 (m, 2H), 2.68-2.76 (m, 1H), 3.10-3.21 (m, 1H), 3.22-3.31 (m, 1H), 3.37 (s, 3H), 3.40 (m, 2H), 3.44-3.56 (m, 5H), 3.60 (m, 2H), 3.68 (m, 1H), 6.91-7.01 (m, 1H); LRMS : m/z 398.7 (M^+)

Example 38

Cis-3-(2-Methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}cyclohexyl)-amino]carbonyl}cyclopentyl)methyl]propanoic acid

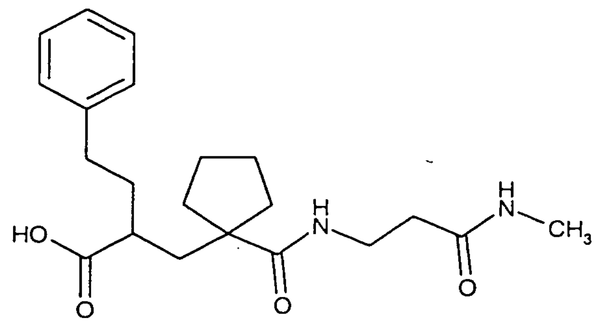


A solution of the *tert*-butyl ester from preparation 66 (446mg, 0.75mmol) in dichloromethane (5ml) and trifluoroacetic acid (5ml) was stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and the residue azeotroped with dichloromethane, then toluene, and finally ether, to afford the title compound as a white foam, 385mg, 95%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.48-2.17 (m, 18H), 2.40 (s, 1H), 2.66 (s, 1H), 3.37 (s, 3H), 3.50-3.70 (m, 6H), 3.94 (s, 1H), 6.10 (d, 1H), 6.59 (s, 1H), 7.55 (t, 2H), 7.61 (m, 1H), 8.02 (d, 2H), 9.11 (s,

1H); Anal. Found: C, 54.88; H, 6.90; N, 5.04. $C_{26}H_{38}N_2O_8S$;1.7H_2O requires C, 57.97; H, 7.11; N, 5.20%.

Example 39

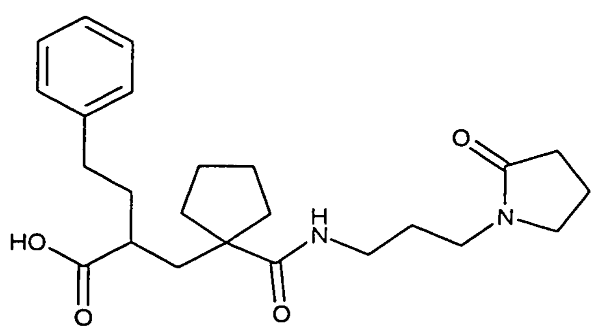
2-{[1-({[3-(Methylamino)-3-oxopropyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 68 (160mg, 0.34mmol) and 10% palladium on charcoal (100mg) in ethanol (30ml) was hydrogenated at room temperature and 60 psi for 18 hours. The mixture was filtered through Arbocel® and the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:acetic acid (95:5:0 to 95:5:0.5) to afford the title compound as a white foam, 100mg, 79%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.40-1.70 (m, 8H), 1.95 (m, 3H), 2.10 (m, 1H), 2.35 (d, 3H), 2.59 (m, 2H), 2.75 (t, 3H), 3.42 (m, 2H), 6.25 (bs, 1H), 6.70 (bs, 1H), 7.13-7.25 (m, 5H); and LRMS: m/z 375.0 (MH^+).

Example 40

2-{[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoic acid.

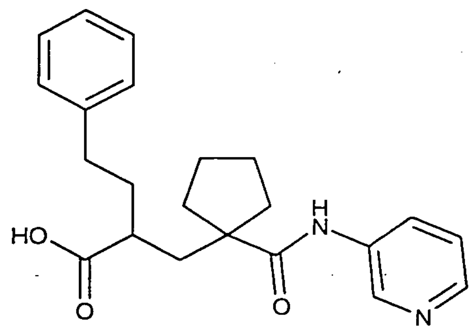


A mixture of the benzyl ester from preparation 67 (780mg, 1.55mmol) and 10% palladium on charcoal (100mg) in ethanol:water (90:10 by volume), (30ml) was

hydrogenated at room temperature under 60psi H_2 pressure for 1.5 hours. The catalyst was filtered off, and the filtrate evaporated under reduced pressure to provide the title compound as a white foam, 473mg, 74%; ^1H NMR ($CDCl_3$, 300MHz) d : 1.26-1.77 (m, 10H), 1.78-2.46 (m, 11H), 2.49-2.70 (m, 2H), 2.95-3.36 (m, 4H), 6.92-7.38 (m, 5H); Anal. Found: C, 64.05; H, 7.73; N, 6.22. $C_{24}H_{34}N_2O_4$;$0.75H_2O$ requires C, 65.88; H, 7.83; N, 6.40%.

Example 41

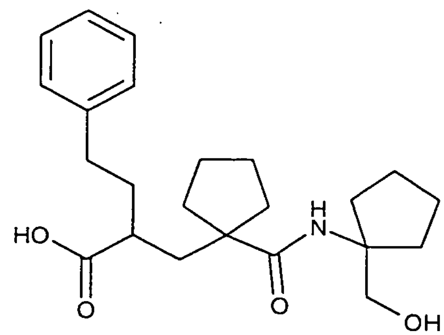
4-Phenyl-2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)butanoic acid



A mixture of the benzyl ester from preparation 71 (700mg, 1.53mmol) and 5% palladium on charcoal (70mg) in ethanol:water (90:10 by volume, 50ml) was hydrogenated at room temperature under 30 psi H_2 pressure for 5 hours. The catalyst was filtered through Arbocel®, washing well with ethanol, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (95:5) as the eluant to provide the title compound as a white foam, 510mg, 91%; mp 80-85°C (collapses to a gum); ^1H NMR ($CDCl_3$, 300MHz) δ: 1.40-2.78 (m, 15H), 6.93-7.39 (m, 5H), 7.93 (m, 1H), 8.59 (d, 1H), 9.17 (d, 1H), 9.41 (s, 1H); Anal. Found: C, 70.83; H, 7.10; N, 7.64. $C_{22}H_{26}N_2O_3$;$0.3H_2O$ requires C, 70.94; H, 7.22; N, 7.52%.

Example 42

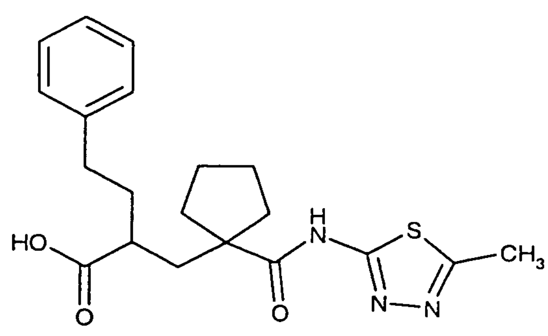
2-{[1-({[1-(Hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 69 (118mg, 0.25mmol) and 10% palladium on charcoal (100mg) in ethanol (20ml) was hydrogenated at room temperature and 60 psi for 18 hours. The mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane to give the title compound as a colourless gum, 95mg, 98%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.41-1.80 (m, 17H), 1.90 (m, 1H), 1.92-2.20 (m, 3H), 2.40 (m, 1H), 2.60 (m, 2H), 3.60 (d, 1H), 3.71 (d, 1H), 5.80 (bs, 1H), 7.15-7.30 (m, 5H); LRMS : m/z 388.1 (MH^+)

Example 43

2-[(1-{[(5-Methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoic acid



A mixture of the benzyl ester from preparation 70 (187mg, 0.39mmol) and 10% palladium on charcoal (80mg) in ethanol (20ml) was hydrogenated at 60 psi for 18 hours. Tlc analysis showed starting material remaining, so additional 10% palladium on charcoal (100mg) was added, and the reaction continued for a further 5 hours. Tlc analysis again showed starting material remaining, so additional catalyst (100mg) was added, and hydrogenation continued for 18 hours. The mixture was filtered

through Arbocel®, and the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane. The crude product was purified by chromatography on silica gel using a Biotage® column, and dichloromethane:methanol (95:5) as eluant to afford the title compound as a clear oil, 80mg, 53%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.51-1.89 (m, 9H), 2.03 (m, 1H), 2.20 (m, 1H), 2.40 (m, 2H), 2.60 (m, 5H), 7.15-7.30 (m, 5H); LRMS : m/z 387.8 (MH^+).

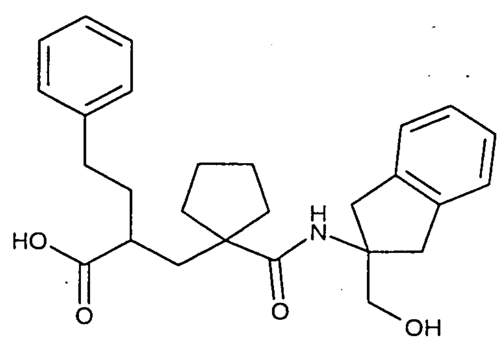
Example 44

(+)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid

and

Example 45

(-)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoic acid



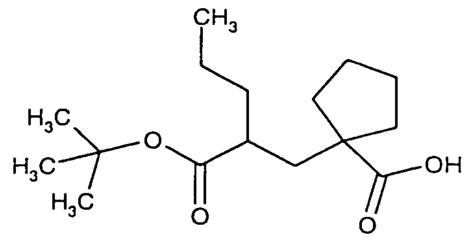
2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (WO 9110644) may be purified by standard HPLC procedures using an AD column and hexane:isopropanol:trifluoroacetic acid (70:30:0.2) as eluant, to give the title compound of example 44, 99.5% ee; $[\alpha]_D = +9.1°$ (c = 1.76 in ethanol); and the title compound of example 45, 99.5% ee; $[\alpha]_D = -10.5°$ (c = 2.2 in ethanol).

The following Preparations describe the preparation of certain intermediates used in the preceding Examples.

NEPi Preparations

Preparation 1

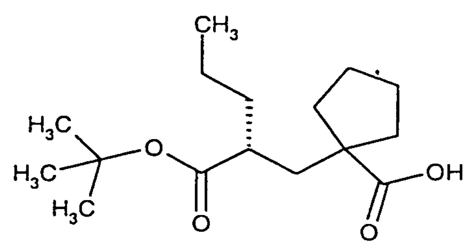
1-[2-(*tert*-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid



A mixture of 1-[2-(*tert*-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic acid (EP 274234) (23g, 81.5mmol) and 10% palladium on charcoal (2g) in dry ethanol (200ml) was hydrogenated at 30psi and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica gel, using ethyl acetate:pentane (40:60) as the eluant, to provide the desired product as a clear oil, 21g, 91%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); LRMS : m/z 283 $(M-H)^-$

Preparation 2

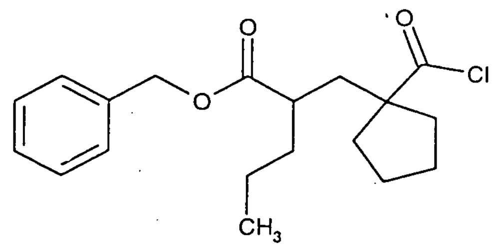
1-[(2*R*)-2-(*tert*-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid



A mixture of (R)-1-[2-(*tert*-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic acid (WO 9113054) (10g, 35.4mmol) and 10% palladium on charcoal (600mg) in dry ethanol (25ml) was hydrogenated at 1 atm. and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give the title compound as a yellow oil, 9.6g, 95%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); $[\alpha]_D = -3.3°$ (c = 0.09, ethanol).

Preparation 3

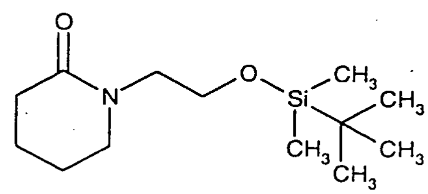
Benzyl 2-{[1-(chlorocarbonyl)cyclopentyl]methyl}pentanoate



Oxalyl chloride (1.15ml, 13.2mmol) was added to an ice-cooled solution of 1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentanecarboxylic acid (EP 274234) (2.0g, 6.3mmol) in dry dichloromethane (20ml), and the solution stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give the title compound as a golden oil, 2.1g; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.28 (m, 2H), 1.43 (m, 2H), 1.63 (m, 6H), 2.00 (m, 1H), 2.08-2.35 (m, 3H), 2.44 (m, 1H), 5.15 (s, 2H), 7.28 (m, 5H).

Preparation 4

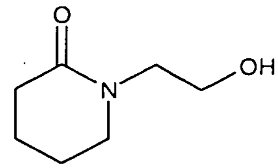
1-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-2-piperidinone



Sodium hydride (807mg, 60% dispersion in mineral oil, 20.18mmol) was added portionwise to a solution of d-valerolactam (2.0g, 20.2mmol) in tetrahydrofuran (100ml) under nitrogen. (2-Bromoethoxy)(*tert*-butyl)dimethylsilane (4.33ml, 20.2mmol) was added portionwise, and the reaction heated at 70°C for 18 hours. Water (50ml) was added to the cooled reaction, the mixture concentrated *in vacuo*, to remove the tetrahydrofuran, and extracted with ethyl acetate (200ml). The organic solution was dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 97:3) to give the title compound, 3.25g; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.00 (s, 6H), 0.83 (s, 9H), 1.75 (m, 4H), 2.35 (m, 2H), 3.39 (m, 4H), 3.75 (t, 2H); LRMS : m/z 257.9 (M^+)

Preparation 5

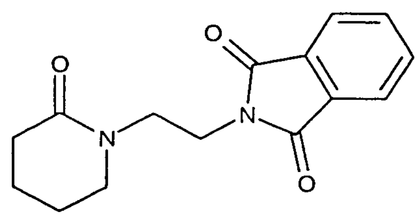
1-(2-Hydroxyethyl)-2-piperidinone



Tetra-n-butylammonium fluoride (14ml, 1M solution in tetrahydrofuran, 14mmol) was added to a solution of the lactam from preparation 4 (3.3g, 12.8mmol) in tetrahydrofuran (50ml), and the reaction stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure, the residue azeotroped with dichloromethane, and purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (97:3 to 95:5) to give the title compound as an oil; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.80 (m, 4H), 2.40 (t, 2H), 3.38 (t, 2H), 3.42 (t, 1H), 3.56 (t, 2H), 3.80 (t, 2H).

Preparation 6

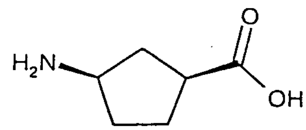
2-[2-(2-Oxo-1-piperidinyl)ethyl]-1*H*-isoindole-1,3(2*H*)-dione



Pthalimide (952mg, 6.47mmol) was added to a solution of the alcohol from preparation 5 (842mg, 5.88mmol) in tetrahydrofuran (30ml), and the mixture sonicated until a solution was obtained. Polymer supported triphenyl phosphine (2.5g, 7.5mmol) and diethyl azodicarboxylate (1.15ml, 7.31mmol) were added, and the reaction stirred at room temperature for 18 hours. The mixture was filtered through Arbocel®, the filtrate concentrated under reduced pressure and the residue azeotroped with dichloromethane. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (70:30 to 100:0), to give the title compound as a white foam, 1.6g (containing some impurities); ^1H NMR ($CDCl_3$, 400MHz) δ: 1.60-1.80 (m, 4H), 2.17 (m, 2H), 3.30 (m, 2H), 3.60 (m, 2H), 3.83 (m, 2H), 7.62 (m, 2H), 7.79 (m, 2H); LRMS : m/z 273.2 (MH^+).

Preparation 7

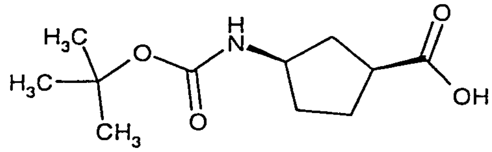
(1*S*,3*R*)-3-Aminocyclopentanecarboxylic acid



Platinum oxide (1g) was added to a solution of (1*R*,4*S*)-4-amino-cyclopent-2-ene carboxylic acid (5.3g, 41.7mmol) in water (70ml), and the mixture was hydrogenated at 45 psi and room temperature for 18 hours. The mixture was filtered through Arbocel®, the filtrate evaporated under reduced pressure, and the residue azeotroped with toluene, to afford the title compound as an off-white solid; ^{1}H NMR (D_2O, 400MHz) δ: 1.70-1.92 (m, 3H), 2.00 (m, 2H), 2.18 (m, 1H), 2.77 (m, 1H), 3.68 (m, 1H); LRMS : m/z 129.8 (MH^+).

Preparation 8

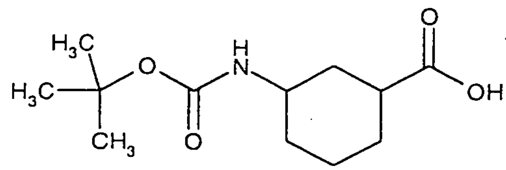
(1*S*,3*R*)-3-[(*tert*-Butoxycarbonyl)amino]cyclopentanecarboxylic acid



Di-*tert*-butyl dicarbonate (10g, 45.8mmol) was added to an ice-cooled solution of the amino acid from preparation 7 (5.4g, 41.8mmol) in dioxan (42.5ml) and sodium hydroxide solution (42.5ml, 1N, 42.5mmol), and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure to remove the dioxan, then acidifed to pH 2 using 2N hydrochloric acid. The aqueous solution was extracted with ethyl acetate (5x100ml), the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure to give a white solid. This was triturated with hexane, to give the desired compound as a crystalline solid, 8.0g, 83%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.58-2.06 (m, 5H), 2.21 (m, 1H), 2.84 (m, 1H), 4.01 (m, 1H), 4.84 (m, 1H); LRMS: m/z 228 ($M-H)^-$.

Preparation 9

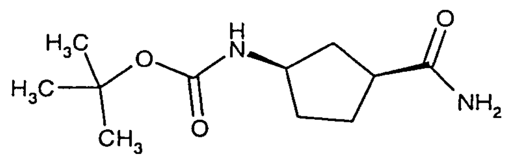
3-[(*tert*-Butoxycarbonyl)amino]cyclohexanecarboxylic acid



The title compound was obtained as a white solid in 81% yield, from 3-aminocyclohexanecarboxylic acid, following the procedure described in preparation 8; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.04 (m, 1H), 1.19-1.50 (m, 13H), 1.83 (m, 1H), 1.97 (m, 2H), 2.24 (m, 1H), 2.40 (m, 1H), 3.44 (bs, 1H), 4.42 (bs, 1H).

Preparation 10

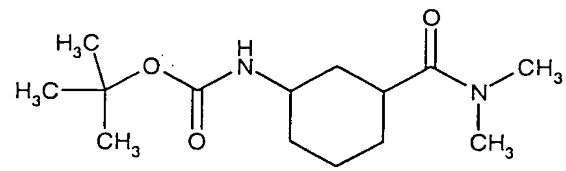
tert-Butyl (1*R*,3*S*)-3-(aminocarbonyl)cyclopentylcarbamate



Benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (3.4g, 6.54mmol), 1-hydroxybenzotriazole hydrate (883mg, 6.54mmol), ammonium chloride (467mg, 8.72mmol) and N-ethyldiisopropylamine (3.04ml, 17.5mmol) were added sequentially to a solution of the acid from preparation 8 (1.0g, 4.37mmol) in N,N-dimethylformamide (16ml), and the reaction stirred at room temperature for 2 hours. The mixture was diluted with ethyl acetate (100ml), washed with water (3x), and brine, then dried ($MgSO_4$) and evaporated under reduced pressure. The residual gum was purified by chromatography on silica gel using a Biotage® column, and an elution gradient of dichloromethane:methanol (98:2 to 95:5). The product was triturated with ether to afford the title compound as a white solid, 438mg, 44%; ^{1}H NMR ($DMSOd_6$, 400MHz) δ: 1.34 (s, 9H), 1.40 (m, 2H), 1.64 (m, 3H), 1.90 (m, 1H), 2.55 (m, 1H), 3.70 (m, 1H), 6.70 (bs, 1H), 6.80 (d, 1H), 7.22 (bs, 1H).

Preparation 11

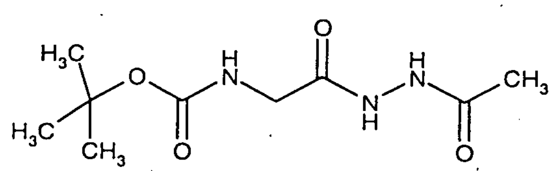
tert-Butyl 3-[(dimethylamino)carbonyl]cyclohexylcarbamate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.19g, 6.19mmol), 1-hydroxybenzotriazole hydrate (840mg, 6.19mmol), N-methylmorpholine (1.1ml, 10.1mmol) and finally 33% ethanolic dimethylamine (1.5ml) were added to a solution of the acid from preparation 9 (1.37g, 5.6mmol) in N,N-dimethylformamide (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure, the residue diluted with ethyl acetate and washed with water (2x). The mixture was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of methanol:dichloromethane (5:95 to 10:90), to give the title compound, 998mg, 66%; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.12 (m, 1H), 1.40 (m, 11H), 1.70 (m, 2H), 1.85 (m, 1H), 2.00 (m, 2H), 2.62 (m, 1H), 2.96 (s, 3H), 3.05 (s, 3H), 3.50 (m, 1H), 4.50 (m, 1H).

Preparation 12

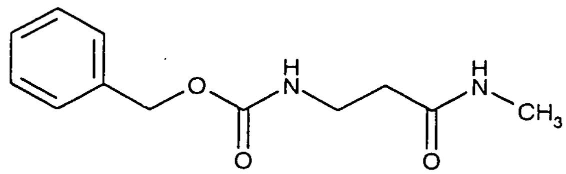
tert-Butyl 2-(2-acetylhydrazino)-2-oxoethylcarbamate



2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (7.06g, 28.5mmol) was added to a solution of N-*tert*-butoxycarbonylglycine (5.0g, 28.6mmol) in dichloromethane (75ml), and the solution stirred for 15 minutes. Acetic hydrazide (2.6g, 35.1mmol) was added, and the reaction stirred at room temperature for 18 hours. The resulting precipitate was filtered off, and dried *in vacuo*, to afford a white crystalline solid, 2.42g. The filtrate was concentrated under reduced pressure, diluted with ether, and the resulting precipitate filtered and dried *in vacuo*, to afford additional product as a white solid, 4.4g, 67% in total; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 2.02 (s, 3H), 3.87 (d, 2H), 5.22 (bs, 1H), 8.27 (bs, 1H), 8.84 (bs, 1H); LRMS : m/z 249.2 (MNH_4^+); Anal. Found: C, 46.41; H, 7.36; N, 17.98, $C_9H_{17}N_3O_4$ requires C, 46.66; H, 7.41; N, 18.13%.

Preparation 13

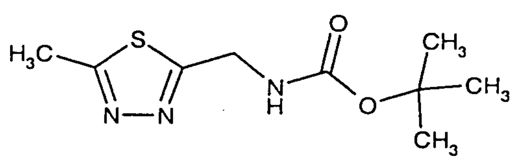
Benzyl 3-(methylamino)-3-oxopropylcarbamate



A mixture of N-[(benzyloxy)carbonyl]-β-alanine (10g, 44.8mmol), methylamine hydrochloride (3.33g, 49.28mmol), 1-hydroxybenzotriazole hydrate (6.05g, 44.8mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10.3g, 53.76mmol) and N-methylmorpholine (11.33ml, 103mmol) in dichloromethane (200ml) was stirred at room temperature for 18 hours. The resulting precipitate was filtered off to give the desired product as a colourless foam, and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:hexane (90:10 to 100:0) to give additional product, 7.96g, 75% in total; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 2.42 (t, 2H), 2.80 (s, 3H), 3.50 (m, 2H), 5.21 (s, 2H), 5.49 (bs, 1H), 5.63 (bs, 1H), 7.36 (m, 5H); Anal. Found: C, 60.68; H, 7.00; N, 11.95. $C_{12}H_{16}N_2O_3$ requires C, 61.00; H, 6.83; N, 11.86%.

Preparation 14

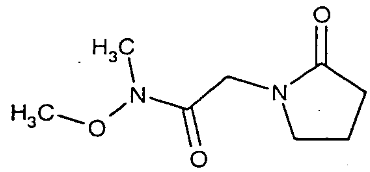
tert-Butyl (5-methyl-1,3,4-thiadiazol-2-yl)methylcarbamate



Lawesson's reagent (960mg, 2.38mmol) was added to a solution of the hydrazide from preparation 12 (500mg, 2.16mmol) in tetrahydrofuran (40ml), and the reaction heated under reflux for 3 hours, then stirred at room temperature for 18 hours. The mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of ethyl acetate:pentane (70:30 to 80:20) to give an oil. This was dissolved in ethyl acetate (100ml), charcoal (2g) added, the mixture stirred for 10 minutes then filtered. The filtrate was concentrated under reduced pressure, and the residue azeotroped with dichloromethane to afford the title compound as a crystalline solid, 441mg, 89%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.45 (s, 9H), 2.77 (s, 3H), 4.66 (d, 2H), 5.22 (bs, 1H); LRMS : m/z 230.1 (MH^+).

Preparation 15

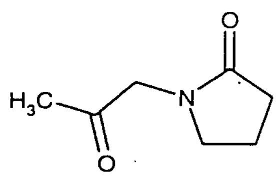
N-Methoxy-*N*-methyl-2-(2-oxo-1-pyrrolidinyl)acetamide



2-Chloro-N-methoxy-N-methylacetamide (3.2g, 23.3mmol) was added to a suspension of 2-pyrrolidinone (2.0g, 23.5mmol) and sodium hydride (940mg, 60% dispersion in mineral oil, 23.5mmol) in tetrahydrofuran (60ml), and the reaction stirred at room temperature for 48 hours. The mixture was quenched with water (150ml), and extracted with ethyl acetate (200ml) and dichloromethane (200ml). The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was triturated with hexane, then ether to afford the title compound as white crystals, 1.8g, 41%; ^1H NMR ($CDCl_3$, 400MHz) δ: 2.02 (m, 2H), 2.40 (t, 2H), 3.17 (s, 3H), 3.48 (t, 2H), 3.72 (s, 3H), 4.19 (s, 2H); LRMS : m/z 186.9 (MH^+).

Preparation 16

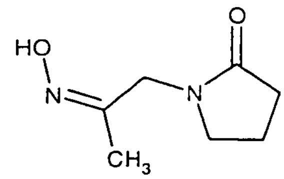
1-(2-Oxopropyl)-2-pyrrolidinone



Methylmagnesium chloride (2.7ml, 3M in tetrahydrofuran, 8.1mmol) was added to a cooled (-20°C) solution of the amide from preparation 15 (1.5g, 8.1mmol) in tetrahydrofuran (50ml), and the reaction allowed to warm to room temperature, then stirred for an hour. The mixture was quenched by the addition of aqueous ammonium chloride solution, then extracted with ethyl acetate (3x50ml). The combined organic solutions were dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as an oil, 645mg, 56%; ^1H NMR ($CDCl_3$, 400MHz) δ: 2.07 (m, 2H), 2.17 (s, 3H), 2.42 (t, 2H), 3.42 (t, 2H), 4.10 (s, 2H).

Preparation 17

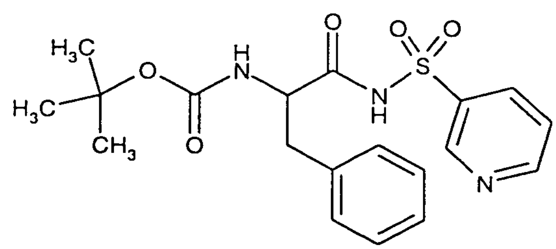
1-[2-(Hydroxyimino)propyl]-2-pyrrolidinone



Hydroxylamine hydrochloride (316mg, 4.55mmol) and then pyridine (370µl, 4.58mmol) were added to a solution of the amide from preparation 16 (643mg, 4.55mmol) in ethanol (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (97:3 to 90:10). The product was triturated with ether to give the title compound as a white solid, 375mg, 53%; ^{1}Hmr (DMSOd$_6$, 400MHz) δ: 1.60 (s, 3H), 1.87 (m, 2H), 2.20 (t, 2H), 3.19 (t, 2H), 3.78 (s, 2H), 10.77 (s, 1H); LRMS : m/z 157.4 (MH$^+$).

Preparation 18

tert-Butyl 1-benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethylcarbamate

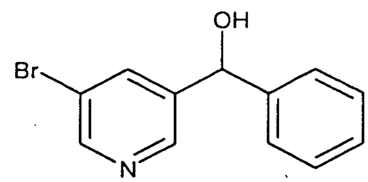


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (939mg, 4.9mmol), 1-hydroxybenzotriazole hydrate (562mg, 4.15mmol), and N-methylmorpholine (952mg, 9.42mmol) were added to an ice-cold solution of N-*tert*-butoxycarbonyl-L-phenylalanine (1.0g, 3.77mmol) in dichloromethane (20ml), and the mixture stirred for 15 minutes. 3-Pyridinesulphonamide (Mon. für Chemie; 72; 77; 1938) (596mg, 3.77mmol) was added, and the reaction stirred at room temperature for 24 hours. The mixture was evaporated under reduced pressure and the residue partitioned between ethyl acetate (50ml) and water (50ml), and the layers separated. The aqueous layer was extracted well with ethyl acetate, then dichloromethane, the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified twice by column chromatography on silica gel, using an elution gradient of ethyl acetate:ethanol (100:0 to 90:10) to give the desired product as a white foam, 1.01g, 66; ^{1}H NMR (DMSOd$_6$, 300MHz) δ: 1.30 (s, 9H), 2.77

(m, 1H), 2.97 (m, 1H), 3.84 (m, 1H), 5.95 (bs, 1H), 6.96 (m, 2H), 7.08 (m, 3H), 7.42 (m, 1H), 8.05 (d, 1H), 8.60 (d, 1H), 8.84 (m, 1H); $[\alpha]_D$ = -10° (0.1% solution in methanol).

Preparation 19

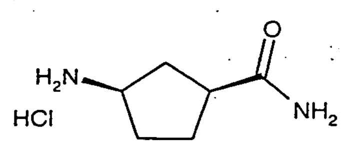
(5-Bromo-3-pyridinyl)(phenyl)methanol



n-Butyl lithium (17ml, 2.5M in hexanes, 42.5mmol) was added dropwise to cooled (-78°C) solution of 3,5-dibromopyridine (10g, 42.2mmol) in ether (200ml), so as to maintain an internal temperature <-70°C. The mixture was then stirred for 15 minutes, and a solution of benzaldehyde (4.5g, 42.5mmol) in ether (20ml) was added dropwise, again maintaining the temperature <-70°C. The mixture was stirred for 15 minutes, then allowed to warm to room temperature over an hour. The reaction was quenched by the addition of 0.9M ammonium chloride solution (200ml), the layers separated, and the aqueous phase extracted with ether. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using an elution gradient of dichloromethane:ether (95:5 to 80:20) to give the title compound as a yellow oil, 7.6g, 68%; ^1H NMR (D_2O, 300MHz) δ: 5.80 (s, 1H), 7.37 (m, 5H), 7.90 (s, 1H), 8.40 (s, 1H), 8.44 (s, 1H).

Preparation 20

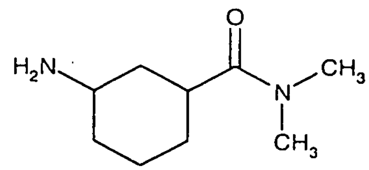
(1*S*,3*R*)-3-Aminocyclopentanecarboxamide hydrochloride



Hydrogen chloride gas was bubbled through an ice-cooled solution of the amide from preparation 10 (438mg, 1.92mmol) in dichloromethane (50ml) for 10 minutes, and the resulting suspension stirred at room temperature for 2 hours. The mixture was purged with nitrogen, then evaporated under reduced pressure. The residue was triturated with ether, to afford the title compound as a solid; ^1H NMR (D_2O, 400MHz) δ: 1.63-1.82 (m, 3H), 1.92-2.07 (m, 2H), 2.19 (m, 1H), 2.82 (m, 1H), 3.62 (m, 1H).

Preparation 21

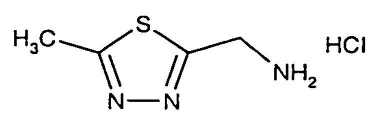
3-Amino-*N*,*N*-dimethylcyclohexanecarboxamide



A solution of the amide from preparation 11 (997mg, 3.69mmol) in trifluoroacetic acid (8ml) and dichloromethane (8ml) was stirred at room temperature for 4 hours. The mixture was concentrated under reduced pressure and the residue partitioned between dichloromethane (25ml) and sodium bicarbonate solution (25ml). The pH was adjusted to 9 using sodium hydroxide solution, the layers separated, and the aqueous phase evaporated under reduced pressure. The resulting solid was triturated with hot ethyl acetate, the suspension filtered and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (84:14:2) to afford the title compound as a colourless oil, 346mg, 55%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.08 (m, 1H), 1.25-1.54 (m, 6H), 1.72 (m, 1H), 1.86 (m, 2H), 2.53-2.75 (m, 2H), 2.96 (s, 3H), 3.03 (s, 3H);

Preparation 22

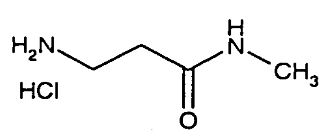
(5-Methyl-1,3,4-thiadiazol-2-yl)methylamine hydrochloride



Hydrogen chloride gas was bubbled through an ice-cooled solution of the thiadiazole from preparation 14 (425mg, 1.85mmol) in dichloromethane (50ml) for 15 minutes, and the reaction stirred at room temperature for 1 hour. The mixture was purged with nitrogen, then evaporated under reduced pressure to afford the title compound as a white solid; ^{1}H NMR ($DMSOd_6$, 400MHz) δ: 2.75 (s, 3H), 4.48 (m, 2H), 8.80 (bs, 3H).

Preparation 23

3-Amino-N-methylpropanamide hydrochloride

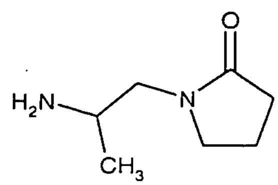


A mixture of the benzyl carbamate from preparation 13 (7.92g, 33.5mmol) and 5% palladium on charcoal (800mg) in ethanol (300ml) was hydrogenated at 50 psi and

room temperature for 4 hours. The reaction mixture was filtered through Arbocel®, washing through with ethanol, and 1N hydrochloric acid (36.9ml, 36.9mmol) was added to the combined filtrate. This solution was evaporated under reduced pressure and the residue azeotroped with dichloromethane to afford the title compound as a colourless foam, 4.66g, ^{1}H NMR (DMSOd$_6$, 300MHz) δ: 2.46 (t, 2H), 2.60 (s, 3H), 2.95 (m, 2H), 7.98-8.16 (m, 2H).

Preparation 24

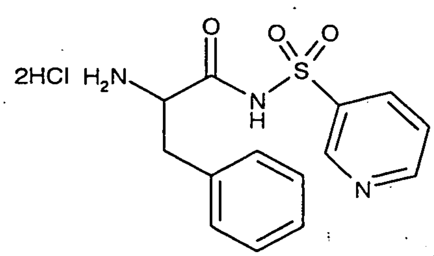
1-(2-Aminopropyl)-2-pyrrolidinone



A mixture of the oxime from preparation 17 (375mg, 2.40mmol) and platinum oxide (300mg) in ethanol (20ml) was hydrogenated at 60psi and room temperature for 18 hours. Tlc analysis showed starting material remaining, so additional platinum oxide (100mg) was added and the reaction continued for a further 4 hours. The mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol:0.88 ammonia (95:5:0.5 to 90:10:1) to give the title compound as a clear oil, 170mg, 50%; ^{1}H NMR (CDCl$_3$, 400MHz) δ: 1.02 (d, 3H), 1.36 (bs, 2H), 2.00 (m, 2H), 2.38 (t, 2H), 3.00-3.16 (m, 2H), 3.21 (m, 1H), 3.35-3.45 (m, 2H); LRMS : m/z 143 (MH$^+$).

Preparation 25

N-(2-Amino-3-phenylpropanoyl)-3-pyridinesulphonamide dihydrochloride

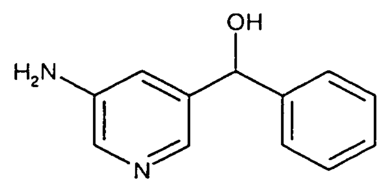


Saturated ethereal hydrochloric acid (40ml) was added to an ice-cold solution of the sulphonamide from preparation 18 (959mg, 2.37mmol) in ethyl acetate (30ml) and ether (10ml), and the solution stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with

dichloromethane (3x) to afford the title compound as a white solid, 959mg; ^1H NMR ($DMSOd_6$, 300MHz) δ: 3.23-3.50 (m, 1H), 3.70-3.98 (m, 1H), 4.13 (m, 1H), 7.05 (m, 2H), 7.20 (m, 3H), 7.78 (m, 1H), 8.36 (d, 1H), 8.44 (bs, 2H), 8.95 (d, 1H), 9.02 (s, 1H);$[\alpha]_D$ = +138° (0.5% solution in methanol).

Preparation 26

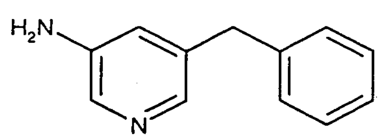
(5-Amino-3-pyridinyl)(phenyl)methanol



A mixture of the bromide from preparation 19 (2.0g, 7.60mmol) and copper (II) sulphate pentahydrate (350mg, 1.40mmol) in 0.88 ammonia (18ml) was heated at 135°C in a sealed vessel for 24 hours. Sodium hydroxide solution (1N, 10ml) was added to the cooled solution, and the mixture was then extracted with ether (6x). The combined organic extracts were dried ($MgSO_4$), and concentrated under reduced pressure to a low volume. The resulting precipitate was filtered, washed with ether and dried to give the title compound as a solid, 1.25g, 83%; mp 92-94°C; ^1H NMR ($DMSOd_6$, 300MHz) δ: 5.22 (s, 2H), 5.59 (d, 1H), 5.86 (d, 1H), 6.83 (s, 1H), 7.20 (m, 1H), 7.34 (m, 4H), 7.78 (m, 2H).

Preparation 27

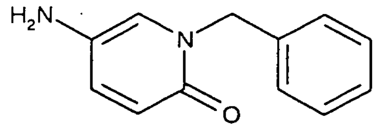
5-Benzyl-3-pyridinylamine



A mixture of the alcohol from preparation 26 (700mg, 3.5mmol) and 5% palladium on charcoal (70mg) in hydrochloric acid (5ml, 1N) and ethanol (20ml) was hydrogenated at 30 psi and room temperature for 6 hours. The mixture was filtered through Arbocel ®, and the filtrate concentrated under reduced pressure. The residue was basified using aqueous sodium bicarbonate solution, extracted with dichloromethane (3x), and the combined organic extracts dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (92:8:0.4) as eluant, to give the title compound as a solid, 500mg, 78%; mp 107-109°C; ^1H NMR ($CDCl_3$, 300MHz) δ: 3.61 (bs, 2H), 3.94 (s, 2H), 6.78 (s, 1H), 7.24 (m, 5H), 7.98 (s, 2H).

Preparation 28

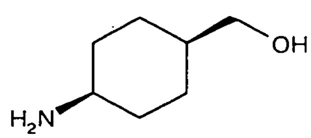
5-Amino-1-benzyl-2(1*H*)-pyridinone



A mixture of 1-benzyl-5-nitro-1H-pyridin-2-one (Justus Liebigs Ann. Chem. 484; 1930; 52) (1.0g, 4.35mmol), and granulated tin (3.5g, 29.5mmol) in concentrated hydrochloric acid (14ml) was heated at 90°C for 1.5 hours. The cooled solution was diluted with water, neutralised using sodium carbonate solution, and extracted with ethyl acetate (250ml in total). The combined organic extracts were filtered, dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as a pale green solid, (turned blue with time), 440mg, 51%; ^1H NMR ($CDCl_3$, 250MHz) δ: 4.12-4.47 (bs, 2H), 5.00 (s, 2H), 6.31 (d, 1H), 6.86 (s, 1H), 7.07 (m, 1H), 7.14-7.42 (m, 5H).

Preparation 29

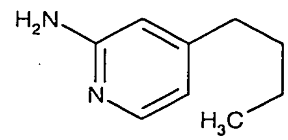
Cis-(4-Aminocyclohexyl)methanol



Lithium aluminium hydride (14ml, 1M solution in tetrahydrofuran, 14mmol) was added dropwise to an ice-cooled solution of cis-4-aminocyclohexanecarboxylic acid (1.33g, 9.29mmol) in tetrahydrofuran (50ml), and once addition was complete, the reaction was heated under reflux for 6 hours. The resulting suspension was cooled to 5°C, and water (0.6ml), aqueous sodium hydroxide solution (1.1ml, 2M), then water (0.6ml) were added sequentially. The resulting suspension was filtered, and the filtrate evaporated under reduced pressure to give an oil, which was used without further purification; ^1H NMR ($CDCl_3$, 300MHz) δ: 1.40-1.80 (m, 12H), 3.00 (m, 1H), 3.55 (d, 2H); LRMS : m/z 130.2 (MH^+).

Preparation 30

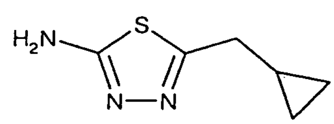
2-Amino-4-butylpyridine



A mixture of 4-butylpyridine (5.0g, 37.0mmol) and 95% sodium amide (1.7g, 40.7mmol) in xylene (10ml) was heated at 150°C for 18 hours. The cooled mixture was diluted with ether (100ml) and extracted with 2N hydrochloric acid (twice). The aqueous extracts were basified using sodium hydroxide solution, and re-extracted with ether. These combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (97:3:0.15) as eluant, to afford the title compound as a crystalline solid, 2.1g, 38%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.96 (t, 3H), 1.38 (m, 2H), 1.60 (m, 2H), 2.52 (t, 2H), 4.38 (bs, 2H), 6.38 (s, 1H), 6.55 (d, 1H), 7.98 (d, 1H); Anal. Found: C, 72.01; H, 9.47; N, 18.53. $C_9H_{14}N_2$ requires C, 71.96; H, 9.39; N, 18.65%.

Preparation 31

5-(Cyclopropylmethyl)-1,3,4-thiadiazol-2-amine

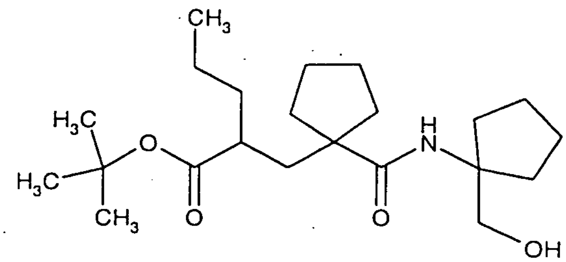


Oxalyl chloride (3.13ml, 35.9mmol) and N,N-dimethylformamide (1 drop) were added to a solution of cyclopropylacetic acid (3g, 29.9mmol) in dichloromethane (30ml), and the reaction stirred at room temperature for 18 hours. The mixture was concentrated under reduced pressure and azeotroped with dichloromethane to give a brown oil.

A mixture of this intermediate acid chloride (887mg, 7.48mmol) and thiosemicarbazide (455mg, 4.99mmol) were heated at 70°C for 18 hours, then cooled. Water was added, the mixture basified to pH 9 using 50% aqueous sodium hydroxide solution, and the resulting precipitate filtered and dried, to give a cream solid, 410mg, 53%; ^{1}H NMR (CD_3OD, 400MHz) δ: 0.28 (m, 2H), 0.60 (m, 2H), 1.02 (m, 1H), 2.77 (d, 2H); LRMS : m/z 155.2 (MH^+).

Preparation 33

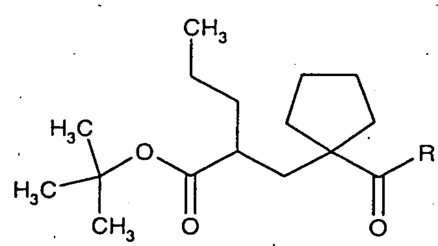
tert-Butyl 2-{[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)-cyclopentyl]methyl}pentanoate



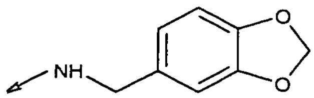
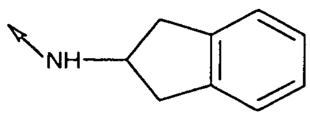
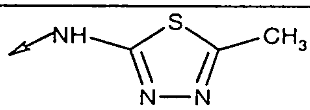
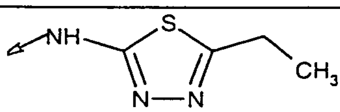
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (41mg, 0.21mmol), 1-hydroxybenzotriazole hydrate (27mg, 0.2mmol), N-methylmorpholine (35μl, 0.31mmol) and finally 1-amino-1-cyclopentanemethanol (25mg, 0.22mmol) were added to a solution of the acid from preparation 1 (150mg, 0.53mmol) in N,N-dimethylformamide (3ml), and the reaction stirred at 90°C for 18 hours. The cooled solution was diluted with ethyl acetate (90ml), washed with water (3x25ml), and brine (25ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using ethyl acetate:pentane (30:70) as the eluant to afford the title compound, 38mg, 57%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.88 (t, 3H), 1.29 (m, 3H), 1.41-1.78 (m, 26H), 1.78-1.98 (m, 4H), 2.04 (m, 1H), 2.26 (m, 1H), 3.59 (dd, 1H), 3.70 (dd, 1H), 4.80 (t, 1H), 5.81 (s, 1H); LRMS : m/z 380 (MH^-).

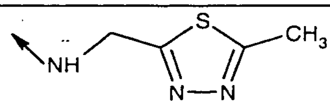
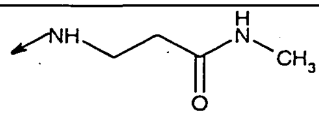
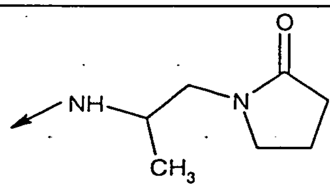
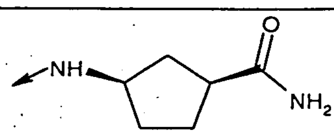
Preparations 34 to 43

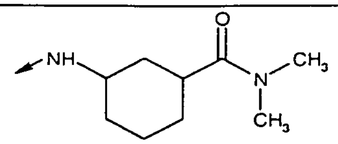
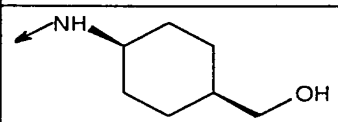
The following compounds of general structure:



were prepared from the acid from preparation 1 and the appropriate amine compound, following a similar procedure to that described in preparation 33.

Prep	R	Starting amine	Yield (%)	Data
34		Piperonylamine	88	[1]H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.26 (m, 4H), 1.42 (s, 9H), 1.46 (m, 2H), 1.59-1.75 (m, 5H), 1.95 (m, 2H), 2.06 (m, 1H), 2.22 (m, 1H), 4.26 (dd, 1H), 4.39 (dd, 1H), 5.95 (m, 3H), 6.78 (m, 3H). LRMS : m/z 418.3 (MH^+)
35[1]		2-Aminoindan hydrochloride	40	[1]H NMR ($CDCl_3$, 400MHz) δ: 0.87 (t, 3H), 1.25 (m, 3H), 4H), 1.42 (m, 12H), 1.56-1.70 (m, 4H), 1.90 (m, 2H), 2.02 (m, 1H), 2.22 (m, 1H), 2.80 (m, 2H), 3.35 (m, 2H), 4.76 (m, 1H), 5.86 (d, 1H), 7.19 (m, 4H). LRMS : m/z 400.3 (MH^+)
36[2]		2-Amino-5-methyl-1,3,4-thiadiazole	76	[1]H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.20-1.85 (m, 20H), 2.18 (m, 4H), 2.67 (s, 3H), 9.80 (bs, 1H). LRMS : m/z 382.3 (MH^+)
37[2]		2-Amino-5-ethyl-1,3,4-thiadiazole	92	[1]H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.20-1.80 (m, 22H), 1.84 (m, 1H), 2.20 (m, 4H), 3.04 (q, 2H), 9.10 (bs, 1H). LRMS : m/z 396.2 (MH^+)

Prep	R	Starting amine	Yield (%)	Data
38		Preparation 22	77	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 4H), 1.42 (s, 9H), 1.44-1.76 (m, 7H), 1.95-2.12 (m, 3H), 2.20 (m, 1H), 2.76 (s, 3H), 4.74 (dd, 1H), 4.82 (dd, 1H), 6.54 (bs, 1H). LRMS : m/z 396.2 (MH^+)
39[1,2]		Preparation 23	60	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.21-1.38 (m, 3H), 1.40-1.70 (m, 17H), 1.88-2.04 (m, 3H), 2.20 (m, 1H), 2.39 (t, 2H), 2.80 (d, 3H), 3.53 (m, 2H), 6.13 (bs, 1H), 6.40 (m, 1H). LRMS : m/z 369.5 (MH^+)
40[2]		Preparation 24	70	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (m, 3H), 1.16 (2xd, 3H), 1.20-1.72 (m, 21H), 1.83 (m, 1H), 1.98 (m, 3H), 2.17 (m, 1H), 2.38 (m, 2H), 1.96 (m, 1H), 3.34 (m, 1H), 3.54-3.62 (m, 2H), 4.15-4.20 (m, 1H), 6.21-6.35 (2xbd, 1H). LRMS : m/z 409.3 (MH^+).
41[2]		Preparation 20	94	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.19-1.38 (m, 4H), 1.42 (m, 12H), 1.60 (m, 3H), 1.74-2.02 (m, 10H), 2.18 (m, 1H), 2.78 (m, 1H), 4.38 (m, 1H), 5.32 (bs, 1H), 5.57 (bs, 1H), 7.28 (bs, 1H). LRMS : m/z 395 (MH^+)

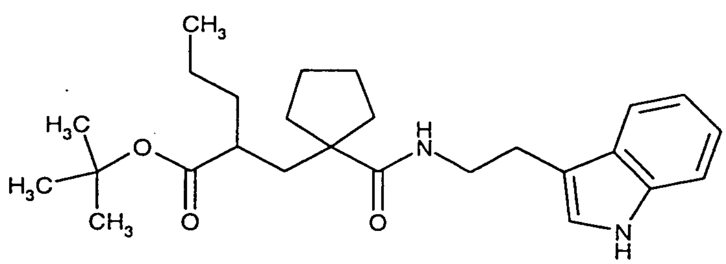
Prep	R	Starting amine	Yield (%)	Data
42[2]		Preparation 21	91	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (t, 3H), 1.18-1.78 (m, 25H), 1.84-2.03 (m, 6H), 2.22 (m, 1H), 2.68 (m, 1H), 2.96 (s, 3H), 3.03 (s, 3H), 3.84 (m, 1H), 5.78 (m, 1H). LRMS : m/z 437.7 (MH^+)
43[2]		Preparation 29	99	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.85 (t, 3H), 1.20-1.79 (m, 30H), 1.90 (m, 2H), 2.05 (m, 1H), 2.24 (m, 1H), 3.56 (m, 2H), 4.04 (m, 1H), 5.82 (bd, 1H). LRMS : m/z 396.4 (MH^+)

1 = reaction conducted at room temperature

2 = Methanol:dichloromethane was used as the column eluant

Preparation 44

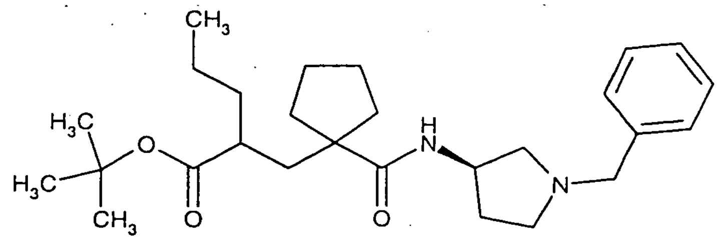
tert-Butyl 2-{[1-({[2-(1*H*-indol-3-yl)ethyl]amino}carbonyl)cyclopentyl]methyl}pentanoate



The title compound was obtained as a pale yellow oil in 80% yield from the acid from preparation 1 and tryptamine, following a similar procedure to that described in preparation 33, except the reaction was performed in dichloromethane at room temperature; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.86 (t, 3H), 1.26 (m, 3H), 1.42 (m, 11H), 1.50-1.69 (m, 6H), 1.83 (m, 1H), 1.90-2.05 (m, 2H), 2.22 (m, 1H), 2.99 (t, 3H), 3.60 (m, 2H), 5.78 (m, 1H), 7.06 (s, 1H), 7.14 (m, 1H), 7.20 (m, 1H), 7.38 (d, 1H), 7.63 (d, 1H), 8.02 (bs, 1H); LRMS : m/z 427.5 (MH^+).

Preparation 45

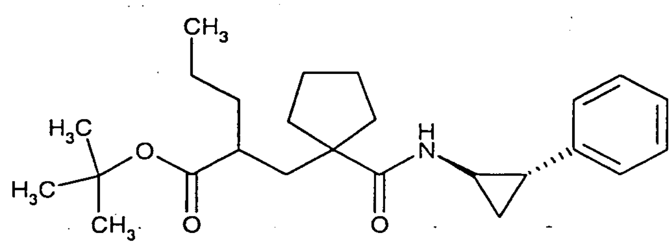
tert-Butyl 2-[(1-{[(3*S*)-1-benzylpyrrolidinyl]amino}cyclopentyl)methyl]pentanoate



The title compound was obtained quantitatively from the acid from preparation 1 and (3S)-1-benzyl-3-aminopyrrolidine, following a similar procedure to that described in preparation 44; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.10-1.76 (m, 21H), 1.90-2.05 (m, 3H), 2.20-2.38 (m, 3H), 2.58 (m, 2H), 2.84 (m, 1H), 3.62 (s, 2H), 4.45 (m, 1H), 6.02 (m, 1H), 7.33 (m, 5H).

Preparation 46

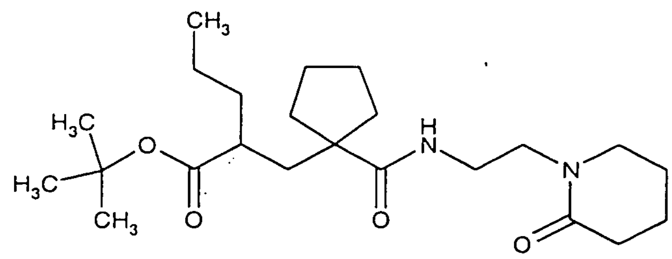
tert-Butyl 2-{[1-({[cis-2-phenylcyclopropyl]amino}carbonyl)-cyclopentyl]methyl}pentanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (81mg, 0.42mmol), N-methylmorpholine (0.15ml, 1.06mmol) and tranylcypromine hydrochloride (60mg, 0.35mmol) were added to a solution of the acid from preparation 1 (100mg, 0.35mmol) in dichloromethane (10ml), and the reaction stirred at room temperature for 18 hours. The reaction mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98:2 to 95:5) to afford the title compound as a yellow oil, 85mg, 55%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (t, 3H), 1.16 (m, 1H), 1.20-1.58 (m, 16H), 1.63 (m, 5H), 1.90-2.14 (m, 4H), 2.23 (m, 1H), 2.90 (m, 1H), 6.00 (m, 1H), 7.19 (m, 3H), 7.24 (m, 2H); LRMS : m/z 400 (MH^+).

Preparation 47

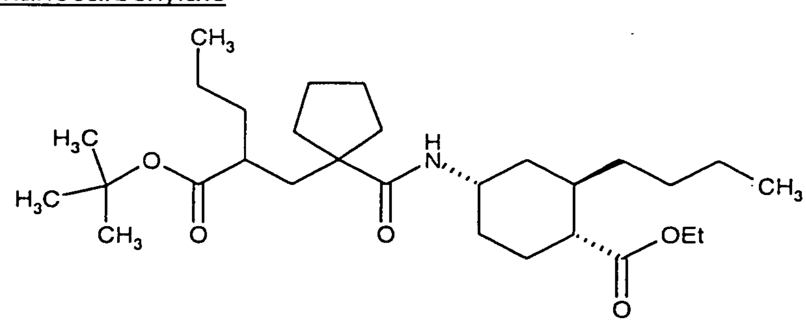
tert-Butyl 2-{[1-({[2-(2-oxo-1-piperidinyl)ethyl]amino}carbonyl)cyclopentyl]-methyl}pentanoate



Hydrazine monohydrate (34µl, 0.70mmol) was added to a solution of the compound from preparation 6 (171mg, 0.63mmol) in ethanol (10ml), and the reaction heated under reflux for 5 hours. The cooled mixture was filtered, the filtrate concentrated under reduced pressure, the residue suspended in dichloromethane, and the suspension re-filtered. The resulting filtrate was concentrated under reduced pressure, and the residue purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (90:10:1) as eluant to give the amine, 16mg. The acid from preparation 1 (32mg, 0.11mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (25mg, 0.13mmol), 1-hydroxybenzotriazole hydrate (17mg, 0.13mmol), and N-methylmorpholine (25µl, 0.23mmol) were added to a solution of this amine in N,N-dimethylformamide (2ml), and the reaction stirred at room temperature for 18 hours. The mixture was partitioned between ethyl acetate and water, and the layers separated. The organc phase was washed with water (2x), dried ($MgSO_4$), and evaporated under reduced pressure. The residual oil was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (98.5:1.5 to 95:5) to afford the title compound as an oil, 43mg, 17%; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.22 (m, 3H), 1.38-1.65 (m, 17H), 1.58 (m, 4H), 1.95 (m, 3H), 2.17 (m, 1H), 2.37 (m, 2H), 3.30 (m, 2H), 3.38 (m, 2H), 3.50 (m, 2H), 6.76 (m, 1H); LRMS : m/z 409.2 (MH^+)

Preparation 48

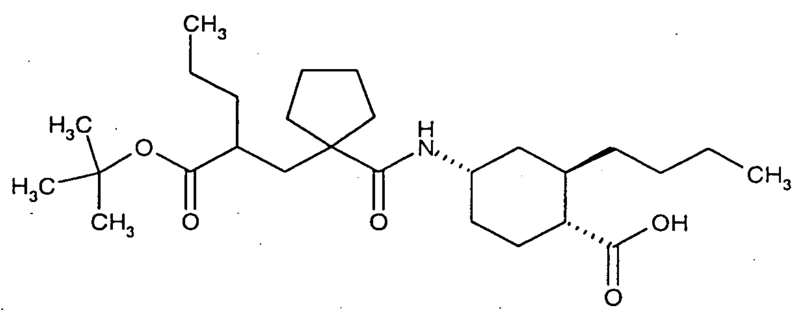
Ethyl (1*R*,2*R*,4*S*)-4-[({1-[2-(*tert*-butoxycarbonyl)pentyl]cyclopentyl}carbonyl)amino]-2-butylcyclohexanecarboxylate



A mixture of the acid from preparation 1 (109mg, 0.38mmol), (1*R*,2*R*,4*S*)-4-amino-2-butyl-cyclohexanecarboxylic acid ethyl ester hydrochoride (WO, 9009374), (101mg, 0.38mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (95mg, 0.50mmol), 1-hydroxybenzotriazole hydrate (60mg, 0.40mmol) and triethylamine (0.12ml, 0.87mmol) in dichloromethane (3ml), was stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue treated with sodium bicarbonate solution and extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (50:50) as eluant, and azeotroped with dichloromethane to afford the title compound, 190mg; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.88 (m, 6H), 1.20-1.40 (m, 13H), 1.40-2.10 (m, 25H), 2.16-2.30 (m, 2H), 4.18 (m, 3H), 5.83 (d, 1H).

Preparation 49

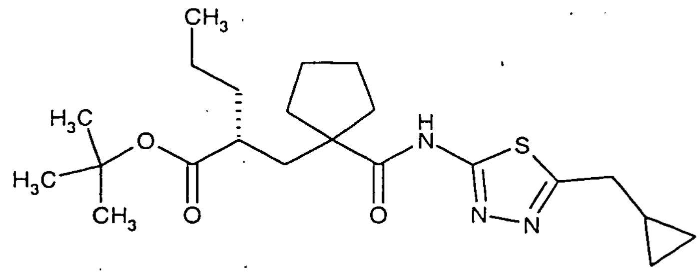
(1*R*, 2*R*,4*S*)-4-[({1-[2-(*tert*-Butoxycarbonyl)pentyl]cyclopentyl}carbonyl)amino]-2-butylcyclohexanecarboxylic acid



A mixture of the ethyl ester from preparation 48 (190mg, 0.39mmol) and 1N sodium hydroxide solution (0.85ml, 0.85mmol) in methanol (1.5ml) was stirred at room temperature for 22 hours. The reaction mixture was acidifed to pH 1 using hydrochloric acid (2N), then partitioned between ethyl acetate and water. The layers were separated, and the organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to afford the title compound, 130mg, 72%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.86 (m, 6H), 1.20-2.12 (m, 36H), 2.24 (m, 2H), 4.18 (m, 1H), 5.82 (d, 1H); LRMS : m/z 464 $(M-H)^-$

Preparation 50

tert-Butyl (2*R*)-2-{[1-({[5-(cyclopropylmethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)cyclopentyl]methyl}pentanoate

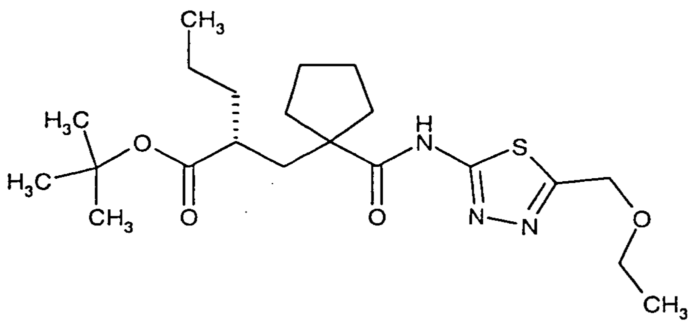


The title compound was prepared from the acid from preparation 2 and the amine from preparation 31, in 65% yield, following the procedure described in preparation 33; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.35 (m, 2H), 0.63 (m, 2H), 0.80 (m, 3H), 1.10 (m, 1H), 1.20-1.94 (m, 20H), 2.19 (m, 4H), 2.93 (t, 2H), 3.50 (s, 1H); LRMS : m/z 422.4 (MH^+)

$[\alpha]_D$ = -14.15° (c = 0.082, methanol).

<u>Preparation 51</u>

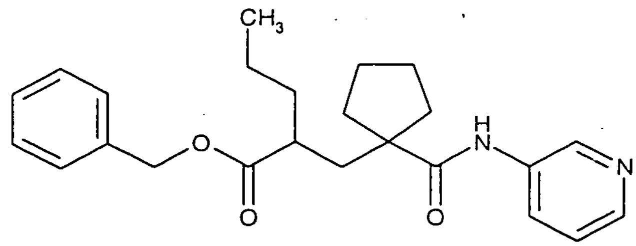
<u>*tert*-Butyl (2*R*)-2-{[1-({[5-(ethoxymethyl)-1,3,4-thiadiazol-2-yl]amino}carbonyl)-cyclopentyl]methyl}pentanoate</u>



The title compound was prepared from the acid from preparation 2 and 5-(ethoxymethyl)-1,3,4-thiadiazol-2-amine, in 51% yield, following the procedure described in preparation 33; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.10-1.78 (m, 25H), 1.82 (m, 1H), 2.19 (m, 5H), 3.48 (s, 1H), 4.82 (s, 2H), 10.16 (brs, 1H); LRMS : m/z 426.4 (MH^+); $[\alpha]_D$ = -12.50° (c = 0.08, methanol).

<u>Preparation 52</u>

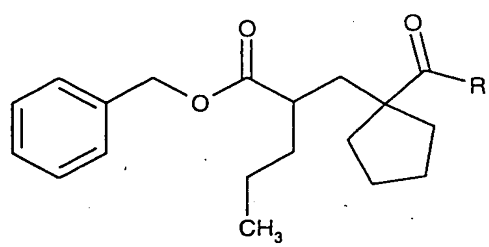
Benzyl 2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoate



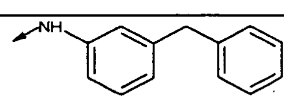
Triethylamine (0.11ml, 0.78mmol) was added to a mixture of the acid chloride from preparation 3 (200mg, 0.60mmol) and 2-aminopyridine (61mg, 0.65mmol) in dichloromethane (3ml), and the reaction stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue partitioned between sodium bicarbonate solution (5ml) and ethyl acetate (20ml), and the layers separated. The organic phase was dried ($MgSO_4$), and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant, to afford the title compound, 130mg; ^1H NMR ($CDCl_3$, 400MHz) δ: 0.82 (t, 3H), 1.21 (m, 3H), 1.40 (m, 1H), 1.43-1.72 (m, 6H), 1.81 (d, 1H), 1.98 (m, 1H), 2.18 (m, 1H), 2.24 (m, 1H), 2.46 (m, 1H), 4.98 (m, 2H), 7.20-7.38 (m, 6H), 7.42 (s, 1H), 8.06 (d, 1H), 8.35 (d, 1H), 8.56 (s, 1H).

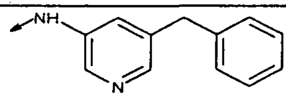
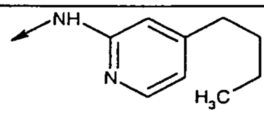
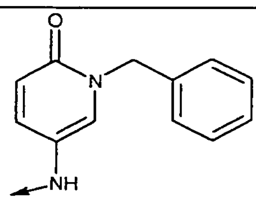
Preparations 53 to 56

The following compounds of general formula:



were prepared from the acid chloride from preparation 3 and the appropriate amine, following a similar procedure to that described in preparation 52.

Prep	R	Yield (%)	Data
53[1]		90	^1H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.24 (m, 2H), 1.40-1.76 (m, 7H), 1.84 (dd, 1H), 1.98 (m, 1H), 2.19 (dd, 1H), 2.28 (m, 1H), 2.56 (m, 1H), 3.98 (s, 2H), 4.99 (dd, 2H), 6.98 (d, 1H), 7.19-7.42 (m, 15H).

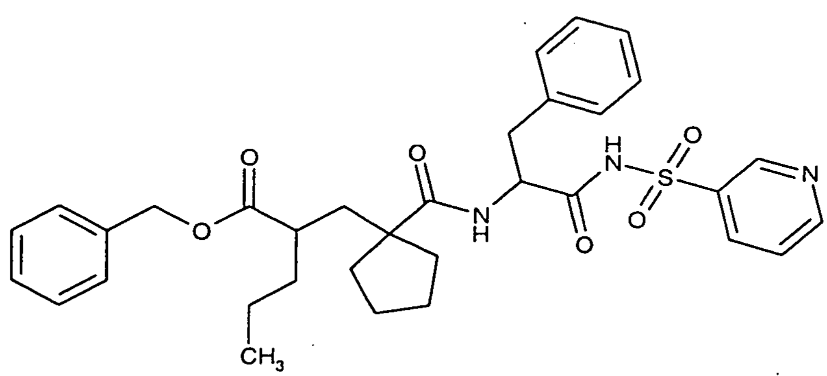
Prep	R	Yield (%)	Data
54		65	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.85 (t, 3H), 1.24 (m, 3H), 1.39-1.78 (m, 6H), 1.82 (dd, 1H), 1.98 (m, 2H), 2.20 (dd, 1H), 2.25 (m, 1H), 2.50 (m, 1H), 3.98 (s, 2H), 4.98 (dd, 2H), 7.18-7.40 (m, 10H), 7.45 (s, 1H), 7.98 (s, 1H), 8.23 (s, 1H), 8.42 (s, 1H).
55		30	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.80 (t, 3H), 0.92 (t, 3H), 1.21 (m, 2H), 1.30-1.70 (m, 12H), 1.82 (dd, 1H), 2.04 (m, 1H), 2.20 (m, 2H), 2.50 (m, 1H), 2.58 (t, 2H), 4.98 (dd, 2H), 6.83 (d, 1H), 7.30 (m, 5H), 7.90 (s, 1H), 8.08 (s, 1H), 8.15 (d, 1H).
56[2]		53	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.25 (m, 2H), 1.27-1.99 (m, 10H), 2.07-2.30 (m, 2H), 2.47 (m, 1H), 4.99 (s, 2H), 5.10 (dd, 2H), 6.59 (d, 1H), 7.15 (d, 1H), 7.34 (m, 11H), 8.10 (s, 1H).

1 = dichloromethane used as the column eluant

2 = N-methylmorpholine was used as the base

Preparation 57

Benzyl 2-({1-[({1-benzyl-2-oxo-2-[(3-pyridinylsulfonyl)amino]ethyl}amino)-carbonyl]cyclopentyl}methyl)pentanoate

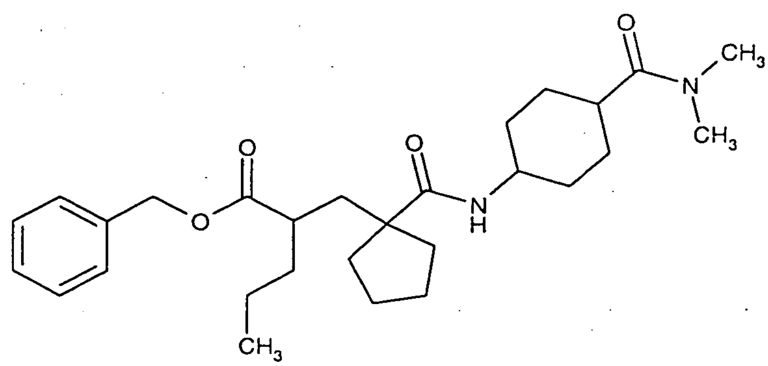


The amine hydrochloride from preparation 25 (828mg, 2.19mmol) and N-methylmorpholine (2.21g, 21.9mmol) was added to an ice-cold solution of the acid chloride from preparation 3 (737mg, 2.19mmol) in dichloromethane (50ml), and the

reaction stirred at room temperature for 24 hours. The reaction mixture was evaporated under reduced pressure, the residue partitioned between ethyl acetate (50ml) and water (50ml), and the layers separated. The organic phase was washed with brine (25ml), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:methanol (100:0 to 95:5) to give the title compound as a cream foam, 975mg, 73%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.72 (m, 3H), 0.94-2.20 (m, 17H), 2.84 (m, 1H), 3.00 (m, 1H), 4.18 (m, 1H), 5.00 (m, 2H), 6.95 (m, 2H), 7.02 (m, 3H), 7.38 (m, 6H), 8.06 (m, 1H), 8.60 (m, 1H), 8.87 (s, 1H).

Preparation 58

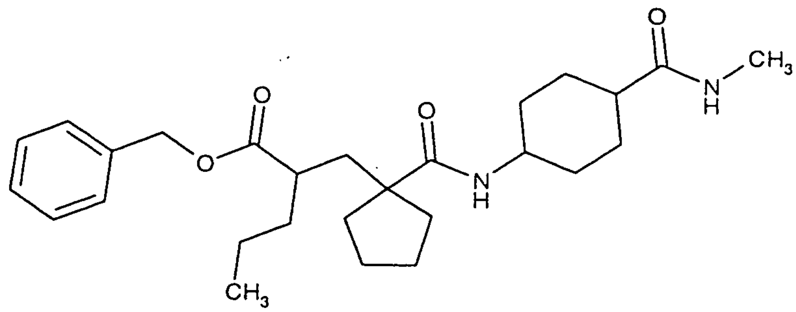
Cis-Benzyl 2-({1-[({4-[(dimethylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoate



A mixture of cis-4-{[(1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentyl)carbonyl]amino}-cyclohexanecarboxylic acid (EP 274234) (200mg, 0.45mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (112mg, 0.58mmol), 1-hydroxybenzotriazole hydrate (70mg, 0.46mmol) and dimethylamine (0.56ml, 2M in tetrahydrofuran, 1.12mmol) in dichloromethane (5ml) was stirred at room temperature for 16 hours. The mixture was concentrated under reduced pressure and the residue partitioned between sodium bicarbonate solution and ethyl acetate, and the layers separated. The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant to afford the title compound, 150mg; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.22 (m, 3H), 1.32-1.88 (m, H), 2.00 (m, 4H), 2.40 (m, 1H), 2.60 (m, 1H), 2.97 (s, 3H), 3.04 (s, 3H), 4.04 (m, 1H), 5.12 (s, 2H), 5.80 (bd, 1H), 7.37 (m, 5H).

Preparation 59

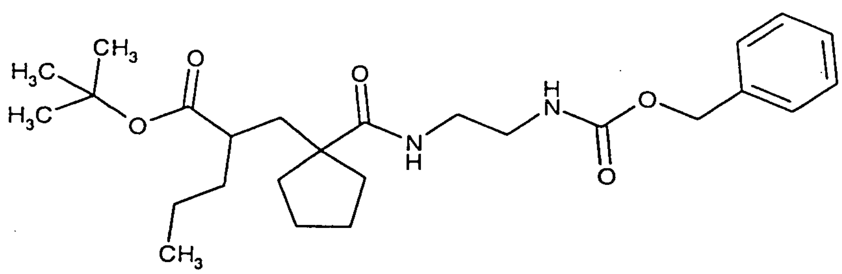
Cis-Benzyl 2-({1-[({4-[(methylamino)carbonyl]cyclohexyl}amino)carbonyl]-cyclopentyl}methyl)pentanoate



The title compound was prepared in 49% yield from cis-4-{[(1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylic acid (EP 274234) and methylamine (2M in tetrahydrofuran), following the procedure described in preparation 58; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.82 (t, 3H), 1.17-2.12 (m, 22H), 2.21 (m, 1H), 2.41 (m, 1H), 2.80 (d, 3H), 4.00 (m, 1H), 5.12 (s, 2H), 5.61 (m, 1H), 5.79 (d, 1H), 7.38 (m, 5H).

Preparation 60

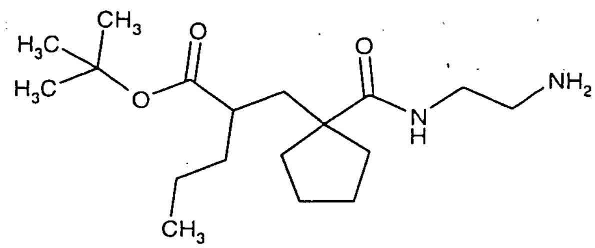
tert-Butyl 2-[(1-{[(2-{[(benzyloxy)carbonyl]amino}ethyl)amino]carbonyl}-cyclopentyl)methyl]pentanoate



The title compound was obtained as a yellow oil in 55% yield, from the acid from preparation 1 and N-benzyloxycarbonyl-1,2-diaminoethane, following a similar procedure to that described in preparation 44; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 3H), 1.40-1.74 (m, 17H), 1.90 (m, 2H), 2.04 (m, 1H), 2.20 (m, 1H), 3.32 (m, 3H), 3.44 (m, 1H), 5.10 (s, 2H), 5.61 (m, 1H), 6.20 (m, 1H), 7.36 (m, 5H).

Preparation 61

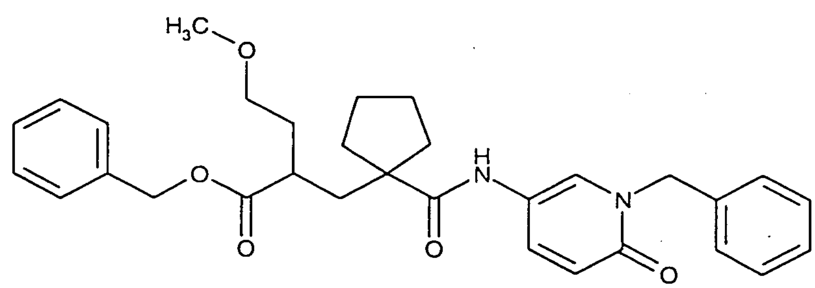
tert-Butyl 2-[(1-{[(2-aminoethyl)amino]carbonyl}cyclopentyl)methyl]pentanoate



A mixture of the carbamate from preparation 60 (1.43g, 3.10mmol) and 10% palladium on charcoal (200mg) in ethanol (8ml) was hydrogenated at room temperature and 1 atm for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to afford the title compound, 920mg, 92%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.84 (t, 3H), 1.20-1.38 (m, 3H), 1.40-1.54 (m, 12H), 1.61 (m, 5H), 1.92-2.12 (m, 3H), 2.20 (m, 1H), 2.98 (m, 2H), 3.38 (m, 1H), 3.42 (m, 1H), 3.97 (m, 2H), 6.65 (m, 1H); LRMS : m/z 326.8 (M^+).

Preparation 62

Benzyl 2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)-methyl]-4-methoxybutanoate

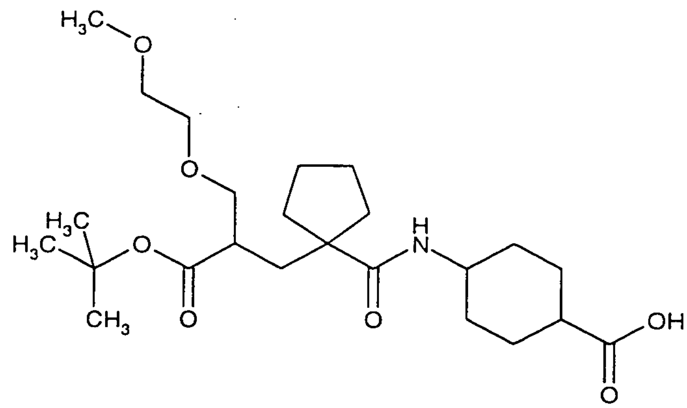


Oxalyl chloride (0.26ml, 3.0mmol) was added to an ice-cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-methoxybutyl}cyclopentanecarboxylic acid (EP 274234) (1.0g, 3.0mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (20ml), and the reaction stirred at room temperature for 2 hours. The solution was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x10ml). The product was dissolved in dichloromethane (20ml), then cooled in an ice-bath. The amine from preparation 28 (600mg, 3mmol) and N-methylmorpholine (0.6ml, 5.45mmol) were added and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and partitioned between water and ether. The organic layer was washed with hydrochloric acid (2N), sodium bicarbonate solution, then water, dried ($MgSO_4$) and evaporated under reduced pressure. The residual green solid was purified by medium pressure column chromatography on silica gel using ethyl acetate:hexane (90:10) as eluant to afford the title compound, 880mg, 57%; ^{1}H NMR ($CDCl_3$,

300MHz) δ: 1.37-2.28 (m, 12H), 2.46-2.64 (m, 1H), 3.20 (s, 3H), 3.31 (m, 2H), 4.97 (dd, 2H), 5.08 (dd, 2H), 6.57 (d, 1H), 7.12 (m, 1H), 7.18-7.48 (m, 10H), 8.08 (d, 1H).

Preparation 63

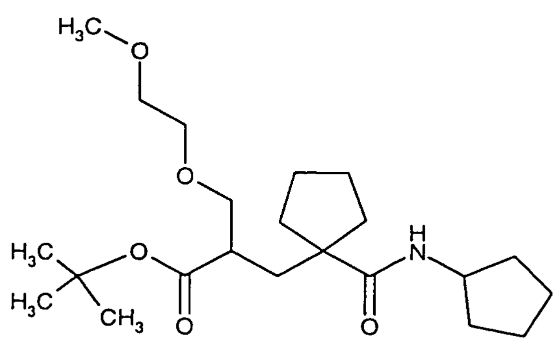
4-{[(1-{3-*tert*-Butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)-carbonyl]amino}cyclohexanecarboxylic acid



A mixture of benzyl 4-{[(1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylate (EP 274234), and 10% palladium on charcoal (250mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 18 hours. The reaction mixture was filtered through Solkafloc®, the filtrate concentrated under reduced pressure and the residue azeotroped with toluene (3x) and then dichloromethane (3x), to give the title compound, 2.0g, 96%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.48 (s, 9H), 1.53-1.84 (m, 14H), 1.94-2.10 (m, 5H), 2.60 (m, 2H), 3.40 (s, 3H), 3.41-3.63 (m, 5H), 3.96 (m, 1H), 5.90 (bd, 1H).

Preparation 64

tert-Butyl 3-{1-[(cyclopentylamino)carbonyl]cyclopentyl}-2-[(2-methoxyethoxy)methyl]-propanoate

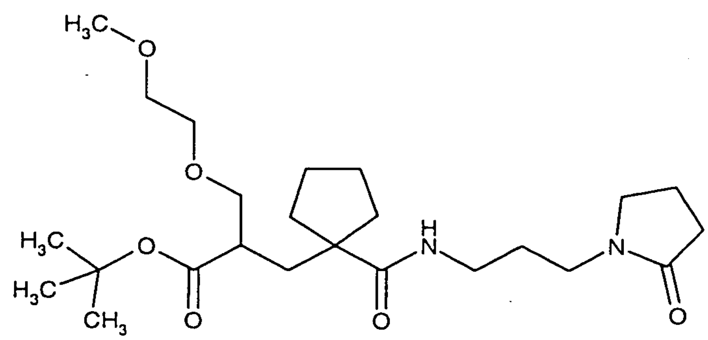


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (197mg, 1.07mmol), 1-hydroxybenzotriazole hydrate (139mg, 1.07mmol), N-methylmorpholine (0.18ml, 1.64mmol) and cyclopentylamine (101μl, 1.07mmol) were added to a solution of 1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}-cyclopentanecarboxylic acid

(EP 274234) (400mg, 1.07mmol) in dichloromethane (5ml), and the reaction stirred at room temperature for 22 hours. The reaction was quenched by the addition of water, extracted with dichloromethane (3x), and the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl acetate:pentane (30:70) as eluant to afford the title compound as a clear oil, 320mg, 78%; ^1H NMR ($CDCl_3$, 400MHz) δ: 1.22-2.02 (m, 27H), 2.58 (m, 1H), 3.36 (s, 3H), 3.40 (m, 1H), 3.46 (m, 2H), 3.57 (m, 3H), 4.10-4.20 (m, 1H), 5.80 (bs, 1H).

Preparation 65

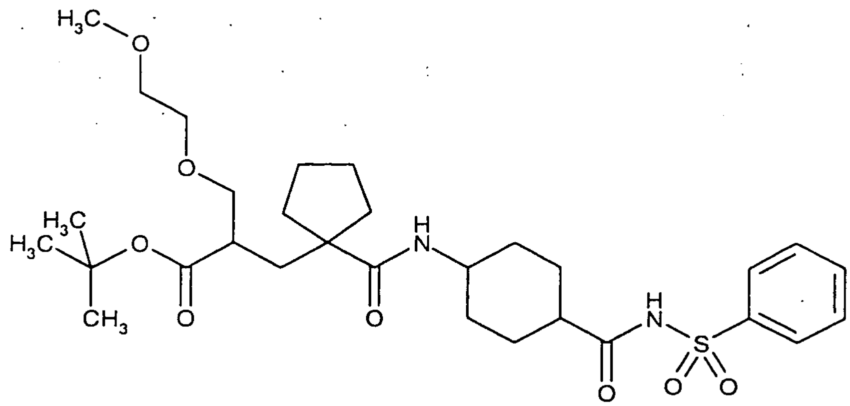
tert-Butyl 3-(2-methoxyethoxy)-2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonyl)cyclopentyl]methyl}propanoate



The title compound was obtained as a clear oil in 97% yield from 1-{3-*tert*-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}-cyclopentanecarboxylic acid (EP 274234) and 1-(3-aminopropyl)-2-pyrrolidinone, following a similar procedure to that described in preparation 64, except dichloromethane:methanol (95:5) was used as the column eluant, ^1H NMR ($CDCl_3$, 400MHz) δ: 1.41 (s, 9H), 1.50 (m, 2H), 1.60-1.70 (m, 7H), 1.78 (m, 1H), 1.90 (m, 1H), 2.20 (m, 4H), 2.40 (m, 2H), 2.58 (m, 1H), 3.14 (m, 1H), 3.20 (m, 1H), 3.38 (m, 6H), 3.42-3.60 (m, 6H), 7.00 (m, 1H).

Preparation 66

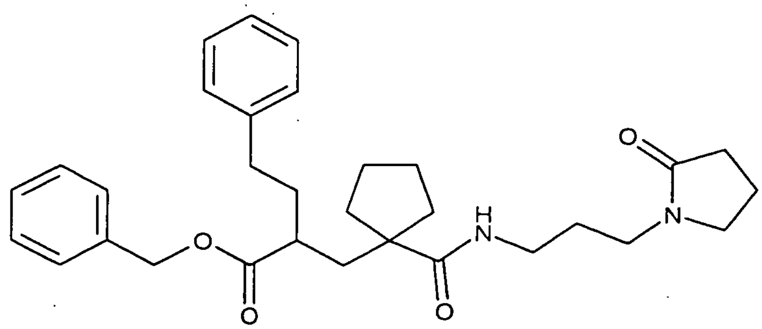
Cis-*tert*-Butyl 3-(2-methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}-cyclohexyl)amino]carbonyl}cyclopentyl)methyl]propanoate



N,N'-Dicyclohexylcarbodiimide (199mg, 0.97mmol), 4-dimethylaminopyridine (118mg, 0.97mmol) and benzenesulphonamide (152mg, 0.97mmol) were added to an ice-cooled solution of the acid from preparation 63 (400mg, 0.878mmol) in dichloromethane (12ml) and N,N-dimethylformamide (0.5ml), and the reaction stirred at room temperature for 20 hours. The mixture was concentrated under reduced pressure and the residue suspended in cold ethyl acetate. The resulting insoluble material was filtered off, the filtrate washed with hydrochloric acid (1N), and water, then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound as a white foam, 480mg, 92%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.44 (s, 9H), 1.63 (m, 13H), 1.80 (m, 2H), 1.88 (m, 1H), 1.98 (m, 2H), 2.36 (m, 1H), 2.57 (m, 1H), 3.38 (s, 3H), 3.40 (m, 1H), 3.51 (t, 2H), 3.58 (m, 3H), 3.95 (m, 1H), 5.92 (d, 1H), 7.56 (m, 2H), 7.62 (m, 1H), 8.05 (d, 2H), 8.75 (bs, 1H); LRMS : m/z 618 (MNa^+).

Preparation 67

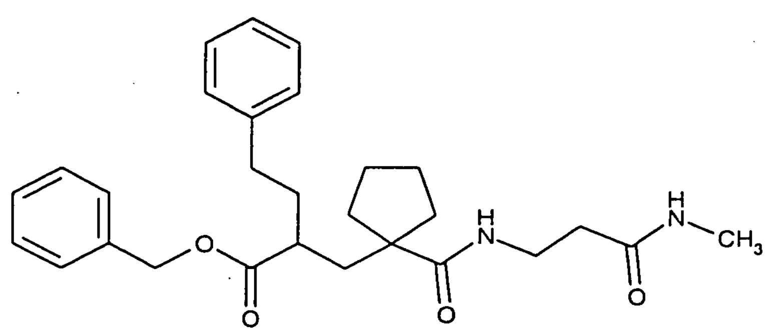
Benzyl 2-{[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.06g, 5.53mmol), 1-hydroxybenzotriazole hydrate (0.60g, 4.44mmol) and 4-methylmorpholine (0.56g, 5.54mmol) were added sequentially to a cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (1.5g, 3.94mmol) in dry dichloromethane (15ml) at room temperature, followed by N-(3-aminopropyl)-2-pyrrolidinone (0.56g, 3.94mmol), and the reaction stirred at room temperature for 18 hours. The mixture was washed with water, 2N hydrochloric acid, saturated aqueous sodium bicarbonate solution, and then dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using ethyl acetate:pentane (50:50) as the eluant to provide the title compound as a clear gum, 800mg, 40%; ^{1}H NMR ($CDCl_3$, 300MHz) d : 1.37-2.20 (m, 16H), 2.34-2.58 (m, 5H), 2.92-3.46 (m, 6H), 5.07 (d, 1H), 5.18 (d, 1H), 6.98-7.47 (m, 10H).

Preparation 68

Benzyl 2-{[1-({[3-(methylamino)-3-oxopropyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoate

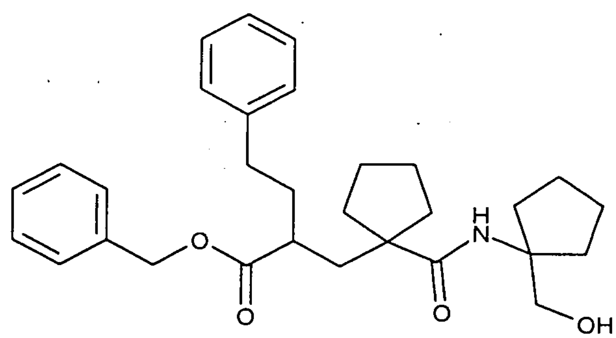


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (122mg, 0.64mmol), 1-hydroxybenzotriazole hydrate (86mg, 0.64mmol) and 4-methylmorpholine (173µl,

1.59mmol) were added sequentially to a cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (202mg, 0.53mmol) in N,N-dimethylformamide (5ml) at room temperature, followed by the amine hydrochloride from preparation 23 (146mg, 1.06mmol), and the reaction stirred at 90°C for 18 hours. The cooled solution was concentrated under reduced pressure and the residue partitioned between water (20ml) and ethyl acetate (100ml). The layers were separated, the organic phase washed with water (3x30ml), brine (25ml) dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (98:2) as eluant to afford the title compound as a colourless oil, 162mg, 67%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.38-1.53 (m, 2H), 1.53-1.96 (m, 8H), 2.02 (m, 2H), 2.27 (t, 2H), 2.46 (m, 3H), 2.76 (d, 3H), 3.44 (m, 2H), 5.13 (s, 2H), 5.79 (bs, 1H), 6.38 (m, 1H), 7.06 (d, 2H), 7.18 (m, 1H), 7.22 (m, 2H), 7.38 (m, 5H); LRMS : m/z 465.5 (MH^+).

<u>Preparation 69</u>

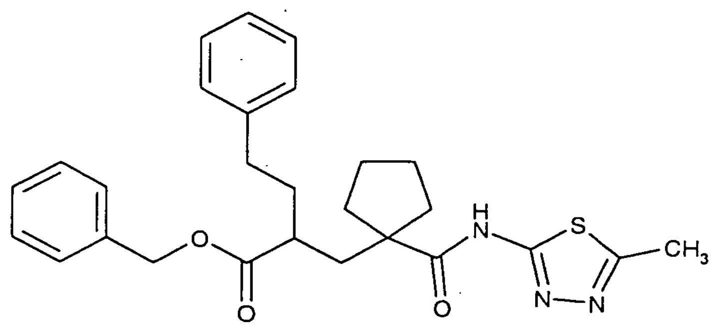
<u>Benzyl 2-{[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoate</u>



The title compound was obtained as a crystalline solid (48%) from 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) and 1-amino-1-cyclopentanemethanol, following a similar procedure to that described in preparation 68, except the reaction mixture was stirred at room temperature for 18 hours, and the crude product purified by column chromatography on silica gel using ethyl acetate:pentane as eluant; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.38 (m, 2H), 1.50-1.95 (m, 16H), 2.01 (m, 2H), 2.45 (m, 3H), 3.49 (dd, 1H), 3.60 (dd, 1H), 4.58 (m, 1H), 5.10 (s, 2H), 5.67 (s, 1H), 7.01 (d, 2H), 7.14 (m, 1H), 7.20 (m, 2H), 7.36 (m, 5H); LRMS : m/z 478.3 (MH^+).

<u>Preparation 70</u>

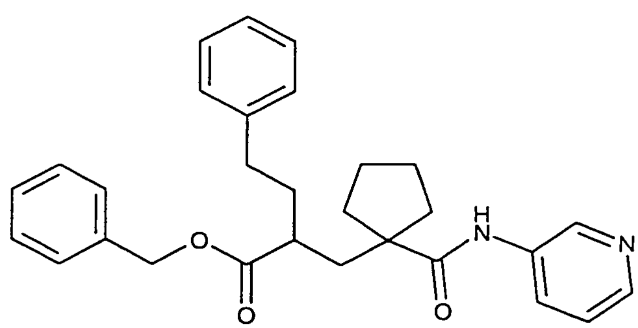
Benzyl 2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoate



The title compound was obtained as a clear oil in 74% yield from 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) and 2-amino-5-methyl-1,3,4-thiadiazole, following a similar procedure to that described in preparation 68; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.58-1.76 (m, 7H), 1.83-1.98 (m, 3H), 2.03 (m, 1H), 2.20 (m, 1H), 2.35 (m, 1H), 2.44 (m, 3H), 2.65 (s, 3H), 5.02 (dd, 2H), 7.00 (d, 2H), 7.15 (m, 1H), 7.19 (m, 2H), 7.35 (m, 5H); LRMS : m/z 478.7 (MH^+).

Preparation 71

Benzyl 4-phenyl-2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)butanoate

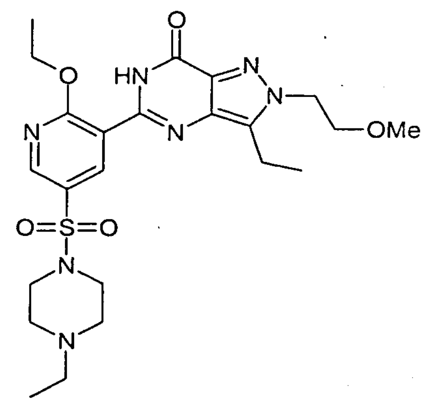


Oxalyl chloride (2.29ml, 26.3mmol) was added to a solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (5.0g, 13.14mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (25ml), and the solution stirred for 2.5 hours. The mixture was evaporated under reduced pressure, the residue azeotroped with dichloromethane to give a yellow oil. This was then dissolved in dichloromethane (50ml) and a solution of this acid chloride (10ml, 2.45mmol) was added to an ice-cooled solution of triethylamine (248mg, 2.45mmol) and 3-aminopyridine (253mg, 2.70mmol) in dry dichloromethane (10ml), and the reaction stirred at room temperature for 18 hours. The solution was washed with water (3x), dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using ethyl

acetate:hexane (40:60) as eluant, and repeated using an elution gradient of ether:hexane (90:10 to 100:0). The product was crystallised from ethyl acetate:hexane to afford the title compound, 740mg, 66%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 1.38-2.07 (m, 10H), 2.10-2.37 (m, 2H), 2.42-2.63 (m, 3H), 5.02 (s, 2H), 6.94-7.44 (m, 10H), 7.50 (s, 1H), 8.03 (d, 1H), 8.36 (d, 1H), 8.52 (s, 1H).

PDE5i Example 1

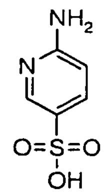
2-(Methoxyethyl)-5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one



A mixture of the product from stage i) below (0.75mmol), potassium bis(trimethylsilyl)amide (298mg, 1.50mmol) and ethyl acetate (73 microlitres, 0.75mmol) in ethanol (10ml) was heated at 120°C in a sealed vessel for 12 hours. The cooled mixture was partitioned between ethyl acetate and aqueous sodium bicarbonate solution, and the layers separated. The organic phase was dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (98:2) as eluant to afford the title compound, 164mg; Found : C, 53.18; H, 6.48; N, 18.14; $C_{23}H_{33}N_7O_5S$;$0.20C_2H_5CO_2CH_3$ requires C, 53.21; H, 6.49; N, 18.25%; δ ($CDCl_3$) : 1.04 (3H, t), 1.40 (3H, t), 1.58 (3H, t), 2.41 (2H, q), 2.57 (4H, m), 3.08 (2H, q), 3.14 (4H, m), 3.30 (3H, s), 3.92 (2H, t), 4.46 (2H, t), 4.75 (2H, q), 8.62 (1H, d), 9.04 (1H, d), 10.61 (1H, s); LRMS : m/z 520 $(M+1)^+$; mp 161-162°C.

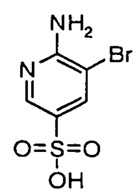
Preparation of Starting Materials for PDE5i Example 1

a) Pyridine-2-amino-5-sulphonic acid



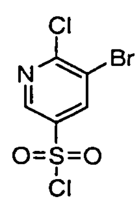
2-Aminopyridine (80g, 0.85mol) was added portionwise over 30 minutes to oleum (320g) and the resulting solution heated at 140°C for 4 hours. On cooling, the reaction was poured onto ice (200g) and the mixture stirred in an ice/salt bath for a further 2 hours. The resulting suspension was filtered, the solid washed with ice water (200ml) and cold IMS (200ml) and dried under suction to afford the title compound as a solid, 111.3g; LRMS : m/z 175 $(M+1)^+$

b) Pyridine-2-amino-3-bromo-5-sulphonic acid



Bromine (99g, 0.62mol) was added dropwise over an hour, to a hot solution of the product from stage a) (108g, 0.62mol) in water (600ml) so as to maintain a steady reflux. Once the addition was complete the reaction was cooled and the resulting mixture filtered. The solid was washed with water and dried under suction to afford the title compound, 53.4g; δ ($DMSOd_6$, 300MHz): 8.08 (1H, s), 8.14 (1H, s); LRMS : m/z 253 $(M)^+$.

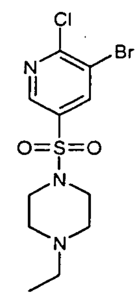
c) Pyridine-3-bromo-2-chloro-5-sulphonyl chloride



A solution of sodium nitrite (7.6g, 110.0mmol) in water (30ml) was added dropwise to an ice-cooled solution of the product from stage b) (25.3g, 100.0mmol) in aqueous hydrochloric acid (115ml, 20%), so as to maintain the temperature below 6°C. The reaction was stirred for 30 minutes at 0°C and for a further hour at room temperature. The reaction mixture was evaporated under reduced pressure and the residue dried under vacuum at 70°C for 72 hours. A mixture of this solid, phosphorus pentachloride (30.0g, 144mmol) and phosphorus oxychloride (1ml, 10.8mmol) was heated at 125°C

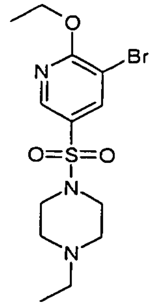
for 3 hours, and then cooled. The reaction mixture was poured onto ice (100g) and the resulting solid filtered, and washed with water. The product was dissolved in dichloromethane, dried ($MgSO_4$), and evaporated under reduced pressure to afford the title compound as a yellow solid, 26.58g; δ ($CDCl_3$, 300MHz) : 8.46 (1H, s), 8.92 (1H, s).

d) <u>3-Bromo-2-chloro-5-(4-ethylpiperazin-1-ylsulphonyl)pyridine</u>



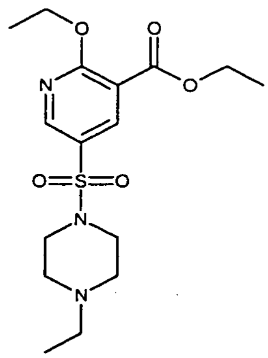
A solution of 1-ethylpiperazine (11.3ml, 89.0mmol) and triethylamine (12.5ml, 89.0mmol) in dichloromethane (150ml) was added dropwise to an ice-cooled solution of the product from stage c) (23.0g, 79.0mmol) in dichloromethane (150ml) and the reaction stirred at 0°C for an hour. The reaction mixture was concentrated under reduced pressure and the residual brown oil was purified by column chromatography on silica gel, using an elution gradient of dichloromethane:methanol (99:1 to 97:3) to afford the title compound as an orange solid, 14.5g; δ ($CDCl_3$, 300MHz) : 1.05 (3H, t), 2.42 (2H, q), 2.55 (4H, m), 3.12 (4H, m), 8.24 (1H, s), 8.67 (1H, s).

e) 3-Bromo-2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridine



A mixture of the product from stage d) (6.60g, 17.9mmol) and sodium ethoxide (6.09g, 89.55mmol) in ethanol (100ml) was heated under reflux for 18 hours, then cooled. The reaction mixture was concentrated under reduced pressure, the residue partitioned between water (100ml) and ethyl acetate (100ml), and the layers separated. The aqueous phase was extracted with ethyl acetate (2x100ml), the combined organic solutions dried ($MgSO_4$) and evaporated under reduced pressure to afford the title compound as a brown solid, 6.41g; Found : C, 41.27; H, 5.33; N, 11.11. $C_{13}H_{20}BrN_3O_3S$ requires C, 41.35; H, 5.28; N, 10.99%; δ ($CDCl_3$, 300MHz) : 1.06 (3H, t), 1.48 (3H, t), 2.42 (2H, q), 2.56 (4H, m), 3.09 (4H, m), 4.54 (2H, q), 8.10 (1H, s), 8.46 (1H, s); LRMS : m/z 378, 380 $(M+1)^+$.

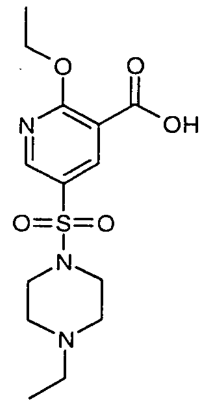
f) Pyridine 2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)-3-carboxylic acid ethyl ester



A mixture of the product from stage e) (6.40g, 16.92mmol), triethylamine (12ml, 86.1mmol), and palladium (0) tris(triphenylphosphine) in ethanol (60ml) was heated at 100°C and 200 psi, under a carbon monoxide atmosphere, for 18 hours, then cooled. The reaction mixture was evaporated under reduced pressure and the residue purified by column chromatography on silica gel, using an elution gradient of dichloromethane:methanol (100:0 to 97:3) to afford the title compound as an orange oil, 6.2g; δ ($CDCl_3$, 300MHz) : 1.02 (3H, t), 1.39 (3H, t), 1.45 (3H, t), 2.40 (2H, q), 2.54

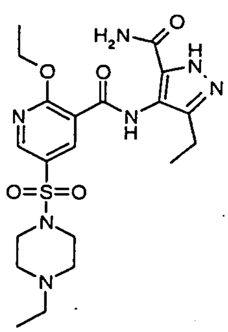
(4H, m), 3.08 (4H, m), 4.38 (2H, q), 4.55 (2H, q), 8.37 (1H, s), 8.62 (1H, s); LRMS : m/z 372 $(M+1)^{+}$

g) Pyridine 2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)-3-carboxylic acid



A mixture of the product from stage f) (4.96g, 13.35mmol) and aqueous sodium hydroxide solution (25ml, 2N, 50.0mmol) in ethanol (25ml) was stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure to half it's volume, washed with ether and acidified to pH 5 using 4N hydrochloric acid. The aqueous solution was extracted with dichloromethane (3x30ml), the combined organic extracts dried ($MgSO_4$) and evaporated under reduced pressure to afford the title compound as a tan coloured solid, 4.02g; δ ($DMSOd_6$, 300MHz) : 1.18 (3H, t), 1.37 (3H, t), 3.08 (2H, q), 3.17-3.35 (8H, m), 4.52 (2H, q), 8.30 (1H, s), 8.70 (1H, s).

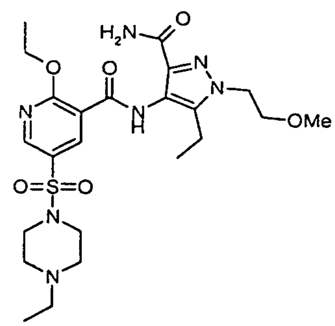
h) 4-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-ylcarboxamido]-1H-3-ethylpyrazole-5-carboxamide



A solution of 4-amino-3-ethyl-1H-pyrazole-5-carboxamide (WO 9849166, preparation 8) (9.2g, 59.8mmol) in N,N-dimethylformamide (60ml) was added to a solution of the product from stage g) (21.7g, 62.9mmol), 1-hydroxybenzotriazole hydrate (10.1g, 66.0mmol) and triethylamine (13.15ml, 94.3mmol) in dichloromethane (240ml). 1-(3-

Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (13.26g, 69.2mmol) was added and the reaction stirred at room temperature for 6 hours. The dichloromethane was removed under reduced pressure, the remaining solution poured into ethyl acetate (400ml), and this mixture washed with aqueous sodium bicarbonate solution (400ml). The resulting crystalline precipitate was filtered, washed with ethyl acetate and dried under vacuum, to afford the title compound, as a white powder, 22g; δ ($CDCl_3$+1 drop $DMSOd_6$) 0.96 (3H, t), 1.18 (3H, t), 1.50 (3H, t), 2.25-2.56 (6H, m), 2.84 (2H, q), 3.00 (4H, m), 4.70 (2H, q), 5.60 (1H, br s), 6.78 (1H, br s), 8.56 (1H, d), 8.76 (1H, d), 10.59 (1H, s), 12.10-12.30 (1H, s); LRMS: m/z 480 $(M+1)^+$.

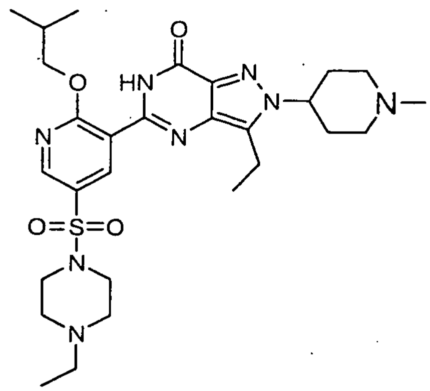
i) <u>2-Methoxyethyl-4-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-ylcarboxamido]-3-ethylpyrazole-5-carboxamide</u>



1-Bromo-2-methoxyethane (1.72mmol) was added to a solution of the product from stage h) (750mg, 1.56mmol) and caesium carbonate (1.12g, 3.44mmol) in N,N-dimethylformamide (15ml) and the reaction stirred at 60°C for 18 hours. The cooled mixture was partitioned between water and ethyl acetate, and the layers separated. The organic layer was dried ($MgSO_4$), concentrated under reduced pressure and azeotroped with toluene to give a solid. This product was recrystallised from ether, to afford the title compound as a white solid.

PDE5i Example 2

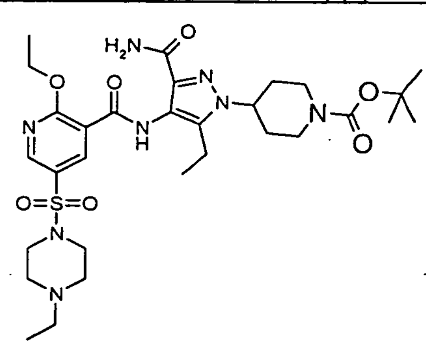
5-[2-*iso*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylpiperidin-4-yl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one



A mixture of the product from stage b) below (90mg, 0.156mmol), potassium bis(trimethylsilyl)amide (156mg, 0.78mmol) and ethyl acetate (14mg, 0.156mmol) in iso-propanol (12ml) was stirred at 130°C for 6 hours in a sealed vessel. The cooled reaction mixture was poured into saturated aqueous sodium bicarbonate solution (60ml), and extracted with ethyl acetate (60ml). The combined organic extracts were dried ($MgSO_4$), and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (92.6:6.6:0.6) to afford the title compound as a beige foam, 36 mg; δ ($CDCl_3$) 1.01 (3H, t), 1.12 (6H, d), 1.39 (3H, t), 1.94 (2H, m), 2.15 (2H, m), 2.22-2.44 (6H, m), 2.55 (6H, m), 3.02 (4H, m), 3.14 (4H, m), 4.22 (1H, m), 4.43 (2H, d), 8.60 (1H, d), 9.00 (1H, d), 10.54 (1H, s).

Preparation of Starting Materials for PDE5i Example 2

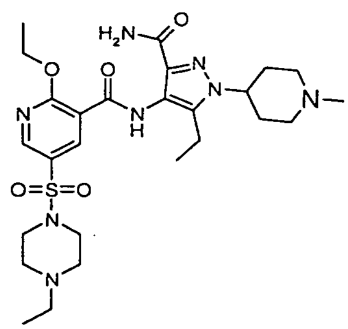
a) 2-(1-*tert*-Butoxycarbonylpiperidin-4-yl)-4-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-ylcarboxamido]-3-ethylpyrazole-5-carboxamide



Sodium hydride (64mg, 60% dispersion in mineral oil, 1.6mmol) was added to a solution of the product from Example 1, stage h) (1.46mmol) in tetrahydrofuran

(10ml), and the solution stirred for 10 minutes. *tert*-Butyl 4-[(methylsulphonyl)oxy]-1-piperidinecarboxylate (WO 9319059) (1.60mmol) was added and the reaction stirred at 60°C for 3 days. The cooled mixture was partitioned between ethyl acetate and aqueous sodium bicarbonate solution, and the phases separated. The aqueous layer was extracted with ethyl acetate, the combined organic solutions dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using dichloromethane:methanol (98:2) as eluant to afford the title compound as a white foam, 310 mg; δ ($CDCl_3$) 1.02 (3H, t), 1.23 (3H, t), 1.49 (9H, s), 1.57 (3H, m), 1.93 (2H, m), 2.16 (2H, m), 2.40 (2H, q), 2.54 (4H, m), 2.82-2.97 (4H, m), 3.10 (4H, m), 4.30 (3H, m), 4.79 (2H, q), 5.23 (1H, s), 6.65 (1H, s), 8.63 (1H, d), 8.82 (1H, d), 10.57 (1H, s).

b) <u>4-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-ylcarboxamido]-3-ethyl-2-(1-methylpiperidin-4-yl)pyrazole-5-carboxamide</u>

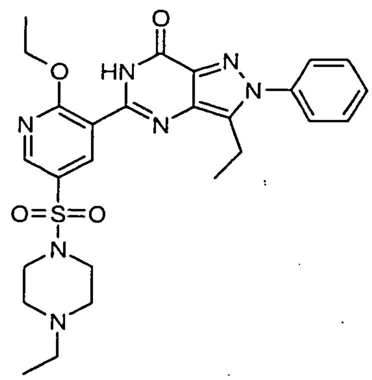


Trifluoroacetic acid (1.5ml) was added to a solution of the product from stage a) above (320mg, 0.48mmol) in dichloromethane (2ml) and the solution stirred at room temperature for 2 ½ hours. The reaction mixture was evaporated under reduced pressure and the residue triturated well with ether and dried under vacuum, to provide a white solid. Formaldehyde (217 microlitres, 37% aqueous, 2.90mmol) was added to a solution of the intermediate amine in dichloromethane (8ml), and the solution stirred vigorously for 30 minutes. Acetic acid (88 microlitres, 1.69mmol) was added, the solution stirred for a further 30 minutes, then sodium triacetoxyborohydride (169mg, 0.80mmol) was added and the reaction stirred at room temperature for 16 hours. The reaction mixture was poured into aqueous sodium bicarbonate solution, and extracted with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (91.75:7.5:0.75) as eluant to afford the title compound, 70mg; δ ($CDCl_3$) 1.02 (3H, t), 1.22 (3H, t), 1.58 (3H, t), 1.92 (2H, m), 2.14

(2H, m), 2.25-2.45 (7H, m), 2.54 (4H, m), 2.91 (2H, q), 2.99-3.16 (6H, m), 4.08 (1H, m), 4.78 (2H, q), 5.11 (1H, br s), 6.65 (1H, br s), 8.63 (1H, d), 8.83 (1H, d), 10.53 (1H, s).

PDE5i Example 3

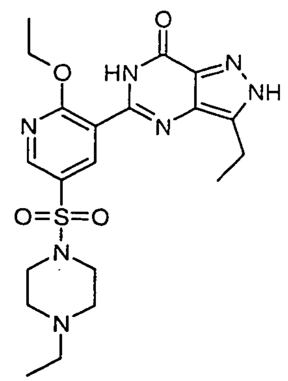
5-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-phenyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one



Pyridine (0.1ml, 1.08mmol) was added to a mixture of the product from stage a) below (250mg, 0.54mmol), copper (II) acetate monohydrate (145mg, 0.72mmol), benzeneboronic acid (132mg, 1.08mmol) and 4Å molecular sieves (392mg) in dichloromethane (5ml), and the reaction stirred at room temperature for 4 days. The reaction mixture was filtered and the filtrate evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (97:3:0.5) as eluant, and triturated with ether:hexane. The resulting solid was filtered and recrystallised from *iso*-propanol:dichloromethane to give the title compound as a solid, 200mg, δ ($CDCl_3$) 1.02 (3H, t), 1.47 (3H, t), 1.60 (3H, t), 2.42 (2H, q), 2.58 (4H, m), 3.10 (2H, q), 3.17 (4H, m), 4.76 (2H, q), 7.40 (1H, m), 7.51 (2H, m), 7.80 (2H, d), 8.67 (1H, d), 9.16 (1H, s), 10.90 (1H, s); LRMS : m/z 538 $(M+1)^+$.

Preparation of Starting Materials for PDE5i Example 3

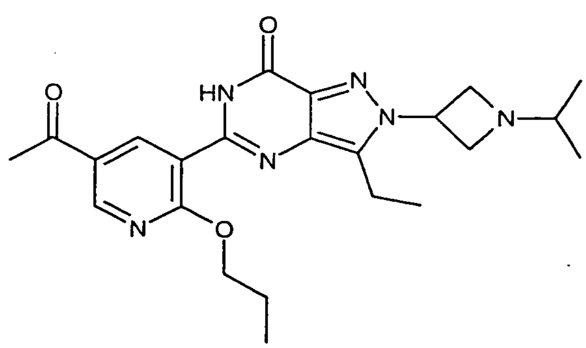
a) 5-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one



Potassium bis(trimethylsilyl)amide (8.28g, 41.6mmol) was added to a solution of the product from Example 1, stage h) (10.0g, 20.8mmol) and ethyl acetate (2ml, 20mmol) in ethanol (160ml), and the reaction mixture heated at 120°C for 12 hours in a sealed vessel. The cooled mixture was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel using dichloromethane:methanol:0.88 ammonia (95:5:0.5) as eluant, to give the title compound, 3.75g; δ ($CDCl_3$) 1.03 (3H, t), 1.42 (3H, t), 1.60 (3H, t), 2.42 (2H, q), 2.58 (4H, m), 3.02 (2H, q), 3.16 (4H, m), 4.78 (2H, q), 8.66 (1H, d), 9.08 (1H, d), 11.00 (1H, s) 11.05-11.20 (1H, br s), LRMS : m/z 462 $(M+1)^+$.

PDE5i Example 4

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-isopropyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one

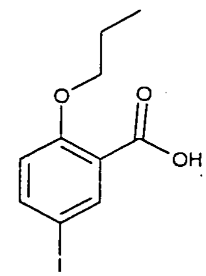


The product from stage h) below (0.23 mmol) was dissolved in dichloromethane (10 ml) and acetone (0.01 ml) was added. After 30 min stirring sodium triacetoxyborohydride (0.51 mmol) was added and stirring continued for 14 h. Further acetone (0.01 ml) and sodium triacetoxyborohydride (0.51 mmol) were added and stirring continued for a further 4.5 h. Starting material still remained so further acetone (0.01 ml) and sodium triacetoxyborohydride (0.51 mmol) were added and stirring continued for a further 18 h. The reaction mixture was diluted with

dichloromethane, washed with sodium bicarbonate solution then brine, dried ($MgSO_4$) and concentrated. Purification by flash column chromatography (elution with 94:6:0.6 dichloromethane/methanol/0.88 ammonia) gave the product as a solid, M.p. 162.8-163.6°C; 1H NMR (400MHz, MeOD): δ = 1.00 (app. d, 9H), 1.30 (t, 3H), 1.84 (app. q, 2H), 2.60 (s, 3H), 2.62-2.72 (m, 1H), 3.00-3.10 (q, 2H), 3.75 (t, 2H), 3.90 (t, 2H), 4.50 (t, 2H), 5.25 (t, 1H), 8.70 (s, 1H), 8.90 (s, 1H); LRMS (TSP – positive ion) 439 (MH^+); Anal. Found C, 61.92; H, 6.84; N, 18.70 Calcd for $C_{23}H_{30}O_3N_6.0.1CH_2Cl_2$: C, 62.07; H, 6.81; N, 18.80.

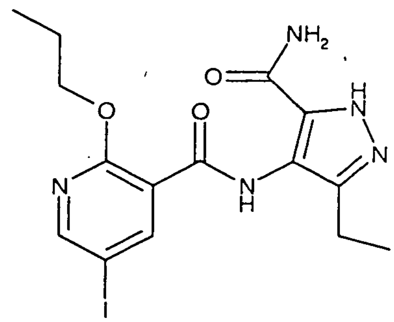
Preparation of Starting Materials for PDE5i Example 4

a) 2-Propoxy-5-iodonicotinic acid



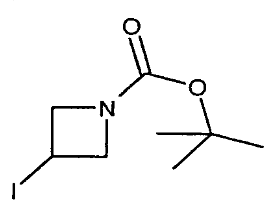
N-Iodosuccinamide (18.22 g, 0.08 mol), trifluoroacetic acid (100 ml) and trifluoroacetic anhydride (25 ml) were added to 2-propoxynicotinic acid (0.054 mol). The mixture was refluxed for 2.5 h, cooled and the solvents evaporated. The residue was extracted from water with ethyl acetate and the organics washed with water (twice) and brine (twice), dried ($MgSO_4$) and concentrated. The red residue was redissolved in ethyl acetate washed with sodium thiosulfate solution (twice), water (twice), brine (twice), redried ($MgSO_4$) and concentrated to give the desired product as a solid; ^1H NMR (300 MHz, $CDCl_3$): δ = 1.05 (t, 3H), 1.85-2.0 (m, 2H), 4.5 (t, 2H), 8.5 (s, 1H), 8.6 (s, 1H); Analysis: found C, 35.16; H, 3.19; N, 4.46. Calcd for $C_9H_{10}INO_3$: C, 35.19; H, 3.28; N, 4.56%.

b) *N*-[3-(Aminocarbonyl)-5-ethyl-1*H*-pyrazol-4-yl]-5-iodo-2-propoxy-nicotinamide



Oxalyl chloride (15.9 mmol) was added to a stirred solution of the product from stage a) (3.98 mmol) in dichloromethane (20 ml) and 3 drops *N,N*-dimethylformamide added. After 2.5 h the solvent was evaporated and the residue azeotroped 3 times with dichloromethane. The residue was resuspended in dichloromethane (4 ml) and added to a stirred mixture 4-amino-3-ethyl-1*H*-pyrazole-5-carboxamide (prepared as described in WO 98/49166) (3.58 mmol) and triethylamine (7.97 mmol) in dichloromethane (10 ml). After 1 h the solvent was evaporated and the residue partitioned between ethyl acetate and water. The organic phase was separated and washed with 2N HCl (twice), sodium bicarbonate solution (twice) and brine before being dried ($MgSO_4$) and concentrated. The product was triturated with ether and filtered to give the title product as a solid. The mother liquor was concentrated and purified by flash column chromatography (elution with 80% ethyl acetate : hexane) to give further product; ^1H NMR (300 MHz, d_4-MeOH): δ = 1.0 (t, 3H), 1.25 (t, 3H), 1.85-2.0 (m, 2H), 2.8 (q, 2H), 4.5 (t, 2H), 8.5 (s, 1H), 8.6 (s, 1H); LRMS (TSP) 444 (MH^+).

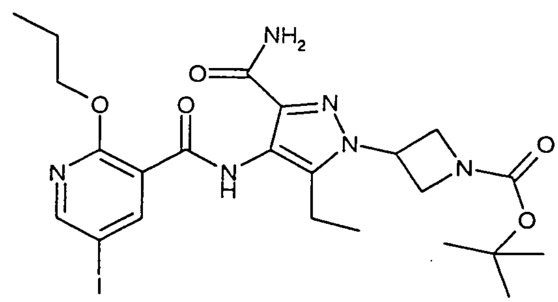
c) <u>*tert*-Butyl 3-iodo-1-azetidinecarboxylate</u>



A mixture of *tert*-butyl 3-[(methylsulfonyl)oxy]-1-azetidinecarboxylate (prepared as described in *Synlett* 1998, 379; 5.0 g, 19.9 mmol), and potassium iodide (16.5 g, 99.4 mmol) in *N,N*-dimethylformamide (25 ml), was heated at 100°C for 42 h. The cooled mixture was partitioned between water and ethyl acetate, and the layers separated. The organic phase was dried over $MgSO_4$, concentrated under reduced pressure and the residue azeotroped with xylene. The crude product was purified by flash column chromatography (dichloromethane as eluant) to give the title compound, 3.26 g; ^1H

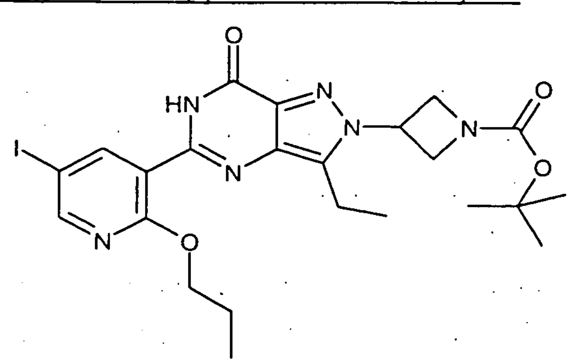
NMR (300 MHz, $CDCl_3$) δ = 1.43 (s, 9H), 4.28 (m, 2H), 4.46 (m, 1H), 4.62 (m, 2H); LRMS (TSP) 284 $(MH)^+$

d) <u>*tert*-Butyl 3-(3-(aminocarbonyl)-5-ethyl-4-{[(5-iodo-2-propoxy-3-pyridinyl)carbonyl]amino}-1*H*-pyrazol-1-yl)-1-azetidinecarboxylate</u>



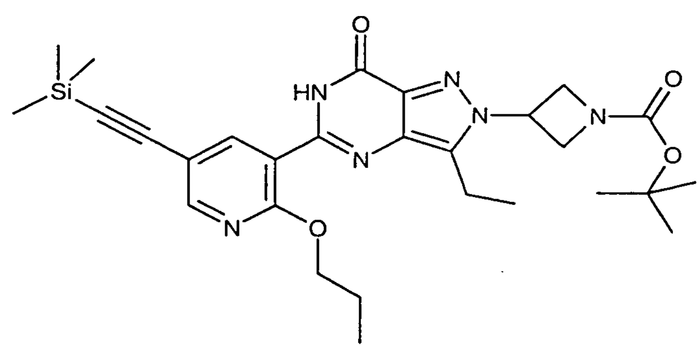
Cesium carbonate (3.59 mmol) was added to a stirred solution of the product from stage b) (1.79 mmol) and the product from stage c) (2.15 mmol) in *N,N*-dimethylformamide (10 ml) under a nitrogen atmosphere. The mixture was heated at 80°C for 24 h. The mixture was cooled and extracted from water with ethyl acetate. The organics were dried ($MgSO_4$) and concentrated to give a brown oil. Purification by flash column chromatography (gradient elution from 100% dichloromethane to 90% dichloromethane/MeOH) gave the title product; 1H NMR (400MHz, DMSO): δ = 0.95 (t, 3H), 1.05 (t, 3H), 1.40 (s, 9H), 1.78-1.88 (m, 2H), 2.68 (q, 2H), 4.22-4.35 (m, 4H), 4.40 (t, 2H), 5.33 (t, 1H), 7.35 (bs, 1H), 7.52 (bs, 1H), 8.40 (s, 1H), 8.55 (s, 1H), 10.10 (s, 1H); LRMS (TSP – positive ion) 373.2 (MH^+ - BOC and I); Anal. Found C, 45.11; H, 5.07; N, 13.56 Calcd for $C_{23}H_{31}O_5N_6I$. 0.2 DCM: C, 45.28; H, 5.14; N, 13.66.

e) <u>*tert*-Butyl 3-[3-ethyl-5-(5-iodo-2-propoxy-3-pyridinyl)-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]-1-azetidinecarboxylate</u>



The product from stage d) (28.4 mmol) was dissolved in n-propanol (200 ml), ethyl acetate (6 ml) and potassium t-butoxide (28.4 mmol) were added and the resultant mixture heated to reflux for 6h. Additional potassium t-butoxide (14.2 mmol) was added and the mixture heated for a further 2h, after which the solvent was removed *in vacuo*. The residue was partioned between water (50 ml) and methylene chloride (100 ml) and the organic phase separated. The aqueous phase was extracted with dichloromethane (2 x 100 ml) and the combined organics dried over $MgSO_4$ and reduced to a solid. Purification by column chromatography (elution with ethyl acetate) gave the title compound; 1H NMR (400MHz, $CDCl_3$): δ = 1.05 (t, 3H), 1.30 (t, 3H), 1.43 (s, 9H), 1.87-1.96 (m, 2H), 3.00 (q, 2H), 4.34 (t, 2H), 4.49 (t, 2H), 4.60 (br s, 2H), 5.20 (t, 1H), 8.41 (d, 1H), 8.94 (s, 1H), 10.75 (br s, 1H); LRMS (TSP – positive ion) 598.1 (MNH_4^+); Anal. Found C, 47.54; H, 5.02; N, 14.09 Calcd for $C_{23}H_{29}O_4N_6I$: C, 47.60; H, 5.04; N, 14.48.

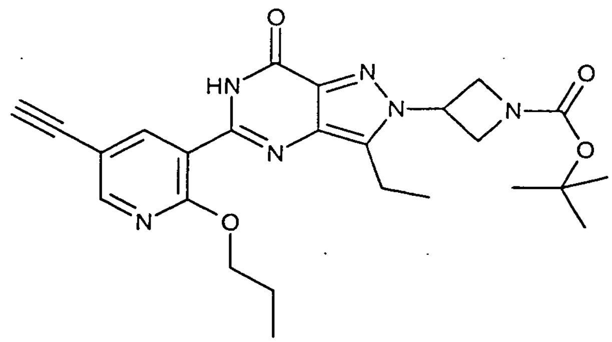
f) <u>*tert*-Butyl 3-(3-ethyl-7-oxo-5-{2-propoxy-5-[(trimethylsilyl)ethynyl]-3-pyridinyl}-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl)-1-azetidinecarboxylate</u>



The product from stage e) (0.25 mmol) was suspended in triethylamine (2 ml) and trimethylsilylacetylene (0.39 mmol) and acetonitrile (2 ml to try and solubilise reactants). $Pd(PPh_3)_2Cl_2$ (0.006 mmol) and cuprous iodide (0.006 mmol) were added and the reaction mixture stirred. After 1 h a further portion of trimethylsilylacetylene (0.19 mmol) was added and stirring continued for 2 h. The solvent was evaporated and the residue partitioned between ethyl acetate and water. The organics were washed with brine, dried ($MgSO_4$) and concentrated. Purification by flash column chromatography (gradient elution from 100% dichloromethane to 99% dichloromethane/methanol) gave the title compound; 1H NMR (400MHz, MeOD): δ = 0.25 (s, 9H), 1.05 (t, 3H), 1.31 (t, 3H), 1.44 (s, 9H), 1.87-1.96 (m, 2H), 3.00 (q, 2H), 4.33 (t, 2H), 4.52 (t, 2H), 4.54-4.80 (m, 2H), 5.18-5.25 (m, 1H), 8.32 (d, 1H), 8.74 (d,

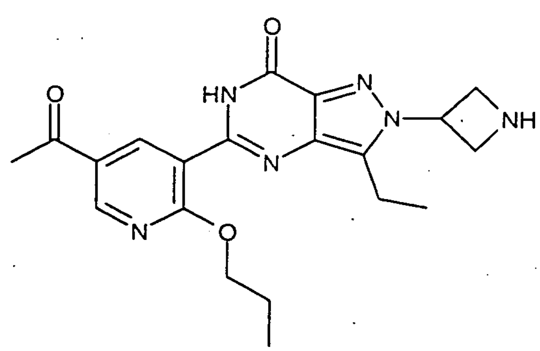
1H); LRMS (TSP – positive ion) 569 (MNH_4^+), 452.0 (MH^+); Anal. Found C, 60.82; H, 6.90; N, 15.15 Calcd for $C_{28}H_{38}O_4N_6Si$: C, 61.07; H, 6.95; N, 15.26.

g) *tert*-Butyl 3-[3-ethyl-5-(5-ethynyl-2-propoxy-3-pyridinyl)-7-oxo-6,7-dihydro-2*H*-pyrazolo[4,3-*d*]pyrimidin-2-yl]-1-azetidinecarboxylate



Potassium fluoride (0.38 mmol) was added to a stirred solution of the product of stage f) (0.19 mmol) in aqueous *N,N*-dimethylformamide (2 ml *N,N*-dimethylformamide /0.2 ml water) at 0°C. After 10 min the reaction was allowed to warm to room temperature and stirred for 2 h. The reaction mixture was diluted with ethyl acetate and washed with water, 1 *N* hydrochloric acid (3 times) and brine. The organic layer was dried ($MgSO_4$) and concentrated to give the title compound as a solid; 1H NMR (400MHz, $CDCl_3$): δ = 1.05 (t, 3H), 1.30 (t, 3H), 1.43 (s, 9H), 1.88-2.00 (m, 2H), 3.00 (q, 2H), 3.19 (s, 1H), 4.35 (app t, 2H), 4.52 (app t, 2H), 4.60-4.80 (br s, 2H), 5.22 (t, 1H), 8.39 (s, 1H), 8.80 (s, 1H), 10.75 (br s, 1H); LRMS (TSP – positive ion) 496 (MNH_4^+).

h) 5-(5-Acetyl-2-propoxy-3-pyridinyl)-2-(3-azetidinyl)-3-ethyl-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one

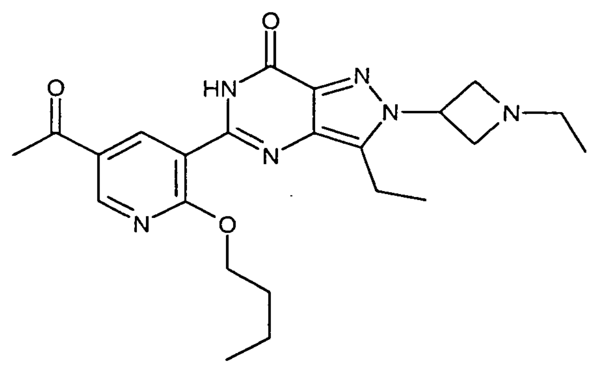


The product from stage g) (1.44 g, 3.0 mmol) in acetone (50 ml) and sulphuric acid (1N, 3 ml) was treated with mercuric sulphate (268 mg, 9.0 mmol) and heated to

reflux for 6h. The reaction mixture was concentrated to ~20 ml *in vacuo*, poured into sodium bicarbonate (sat. aq., 20ml) and extracted into methylene chloride (6 x 20 ml). Combined organics were washed with brine (20 ml), dried over $MgSO_4$, and concentrated to a brown oil which was taken up in 40% trifluoroacetic acid in methylene chloride (50ml) and water (1 ml) and stirred for 1h at room temperature. After evaporation *in vacuo*, the residue was purified by column chromatography (eluting with 95:5:1 methylene chloride:methanol:0.88 ammonia) to afford the title compound as a white hydroscopic foam (1.65 g); m.p. 128.5-130.0°C; 1H NMR (400MHz, MeOD): δ = 1.00 (t, 3H), 1.30 (t, 3H), 1.79-1.90 (m, 2H), 2.60 (s, 3H), 3.00-3.10 (q, 2H), 4.50 (t, 2H), 4.60-4.70 (m, 4H), 5.65-5.78 (m, 1H), 8.65 (s, 1H), 8.90 (s, 1H); LRMS (TSP – positive ion) 397 (MH^+).

PDE5i Example 5

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one

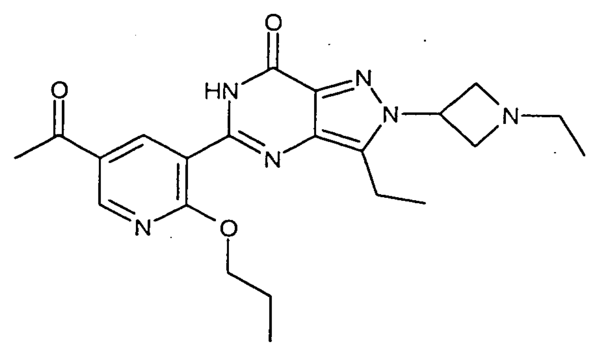


The starting material (120 mg, 0.28 mmol) and cesium carbonate (274 mg, 0.84 mmol) were dissolved in *n*-butanol (4 ml), and heated at 90°C under nitrogen with molecular sieves for 96h. The mixture was then partitioned between water (10 ml) and dichloromethane (10 ml). The organic layer was separated, and the aqueous layer extracted further with dichloromethane (3 x 15 ml). The combined organic layers were dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash column chromatography (95:5:0.5-90:10:1 ethyl acetate:methanol:0.88 NH_3 as eluents), to yield the title compound as a colourless glass (77 mg, 0.18 mmol); m.p. 91.6-93.7°C; 1H NMR (400MHz, $CDCl_3$): δ = 1.00-1.05 (m, 6H), 1.38 (t, 3H), 1.50-1.62 (m, 2H), 1.90-2.00 (m, 2H), 2.63 (s, 3H), 2.63-

2.70 (m, 2H), 3.02 (q, 2H), 3.75 (t, 2H), 3.90 (t, 2H), 4.68 (t, 2H), 5.10-5.20 (m, 1H), 8.84 (s, 1H), 9.23 (s, 1H), 10.63 (br s, 1H); LRMS (TSP – positive ion) 439 (MH^+); Anal. Found C, 60.73; H, 7.06; N, 18.03 Calcd for $C_{23}H_{30}O_3N_6$.0.2MeOH.0.1 DIPE: C, 60.88; H, 7.26; N, 17.90.

Preparation of Starting Materials for PDE5i Example 5

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one



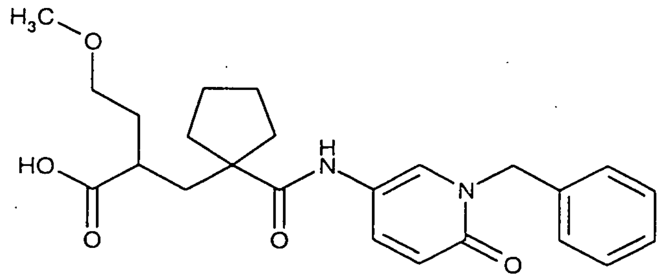
Sodium cyanoborohydride (92 mg, 1.47 mmol) was added to a stirred solution of the product from Example 4 stage h) (500 mg, 0.98 mmol) and sodium acetate (161 mg, 1.96 mmol) in methanol (10 ml) under nitrogen at room temperature. After 1h the mixture was poured into $NaHCO_3$ (sat. aq., 20 ml), and extracted with dichloromethane (3 x 15 ml). The combined organic layers were dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (95:5:0.5-80:20:1 ethyl acetate:methanol:0.88 NH_3 as eluent) to yield the title compound as a white solid (140 mg, 0.33 mmol); 1H NMR (400MHz, $CDCl_3$): δ = 0.97 (t, 3H), 1.03 (t, 3H), 1.30 (t, 3H), 2.82-2.97 (m, 2H), 2.58-2.65 (m, 5H), 2.98 (q, 2H), 3.68 (t, 2H), 3.85 (dd, 2H), 4.58 (dd, 2H), 5.05-5.17 (m, 1H), 8.79 (s, 1H), 9.18 (s, 1H), 10.62 (br s, 1H); LRMS (TSP – positive ion) 426 (MH^+).

Further NEP Chemical Compound Examples

In the following commentary, the Preparation Examples are the synthesis of intermediates; whereas the Examples are the synthesis of the respective, compounds of the present invention.

Example 1

2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)methyl]-4-methoxybutanoic acid (F57)

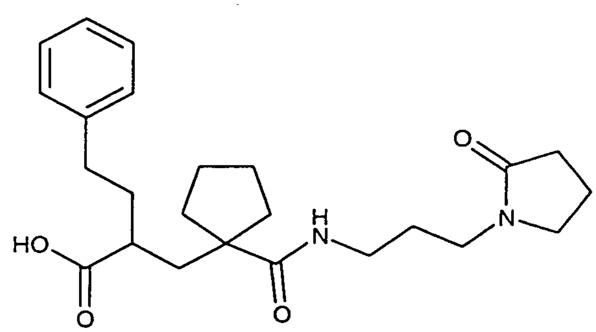


A mixture of the benzyl ester from preparation 1 (1/62) (850mg, 1.64mmol), and 5% palladium on charcoal (250mg) in 40% aqueous ethanol (21ml), was hydrogenated at 30 psi and room temperature for 30 minutes. The reaction mixture was filtered through Hyflo®, and the filtrate evaporated under reduced pressure. The residual foam was purified by column chromatography on silica gel using dichloromethane:methanol (97:3) as eluant to give the title compound as a white foam, 550mg, 79%; ^{1}H NMR (DMSO-d_6, 300MHz) d: 1.24-2.17 (m, 12H), 2.18-2.31 (m, 1H), 3.07 (s, 3H), 3.21 (t, 2H), 5.08 (s, 2H), 6.63 (d, 1H), 7.23-7.41 (m, 5H), 7.72 (d, 1H), 8.24 (s, 1H).

Anal. Found: C, 67.46; H, 7.18; N, 6.24. $C_{24}H_{30}N_2O_5$ requires C, 67.58; H, 7.09; N, 6.57%.

Example 2

2-{[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoic acid. (F58)

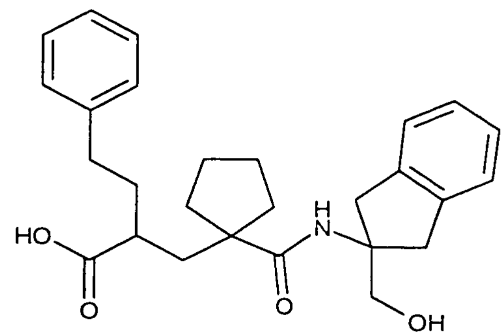


A mixture of the benzyl ester from preparation 3 (3/67) (780mg, 1.55mmol) and 10% palladium on charcoal (100mg) in ethanol:water (90:10 by volume), (30ml) was hydrogenated at room temperature under 60psi H_2 pressure for 1.5 hours. The

catalyst was filtered off, and the filtrate evaporated under reduced pressure to provide the title compound as a white foam, 473mg, 74%; ^{1}H NMR ($CDCl_3$, 300MHz) d : 1.26-1.77 (m, 10H), 1.78-2.46 (m, 11H), 2.49-2.70 (m, 2H), 2.95-3.36 (m, 4H), 6.92-7.38 (m, 5H); Anal. Found: C, 64.05; H, 7.73; N, 6.22. $C_{24}H_{34}N_2O_4$;$0.75H_2O$ requires C, 65.88; H, 7.83; N, 6.40%.

Example 3

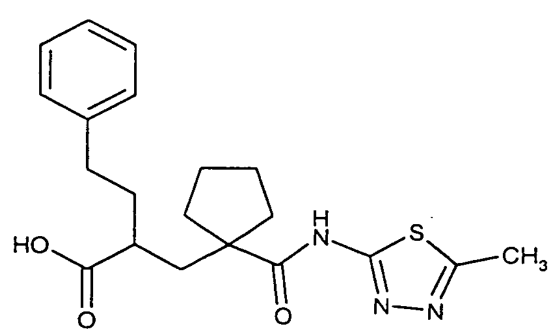
(+)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (F59)



2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (WO 9110644) may be purified by standard HPLC procedures using an AD column and hexane:isopropanol:trifluoroacetic acid (70:30:0.2) as eluant, to give the title compound of example 3, 99.5% ee; $[\alpha]_D = +9.1°$ (c = 1.76 in ethanol)

Example 4

2-[(1-{[(5-Methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoic acid (F60)

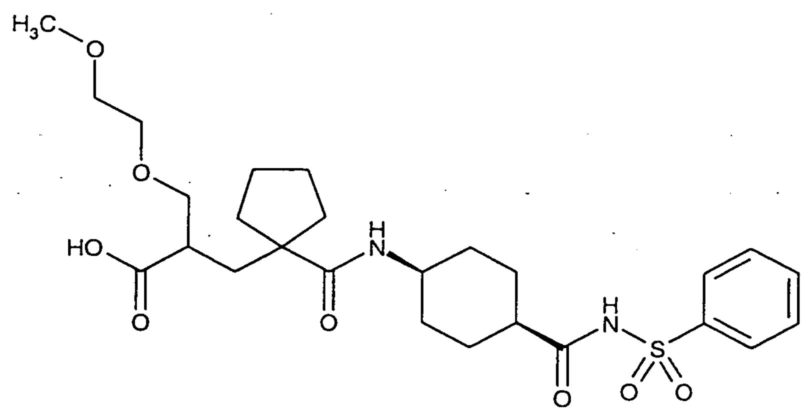


A mixture of the benzyl ester from preparation 4 (4/70) (187mg, 0.39mmol) and 10% palladium on charcoal (80mg) in ethanol (20ml) was hydrogenated at 60 psi for 18 hours. Tlc analysis showed starting material remaining, so additional 10% palladium on charcoal (100mg) was added, and the reaction continued for a further 5 hours. Tlc analysis again showed starting material remaining, so additional catalyst (100mg) was added, and hydrogenation continued for 18 hours. The mixture was filtered

through Arbocel®, and the filtrate concentrated under reduced pressure, and azeotroped with dichloromethane. The crude product was purified by chromatography on silica gel using a Biotage® column, and dichloromethane:methanol (95:5) as eluant to afford the title compound as a clear oil, 80mg, 53%; ^{1}H NMR ($CDCl_3$, 300MHz) d: 1.51-1.89 (m, 9H), 2.03 (m, 1H), 2.20 (m, 1H), 2.40 (m, 2H), 2.60 (m, 5H), 7.15-7.30 (m, 5H); LRMS : m/z 387.8 (MH^+).

Example 5

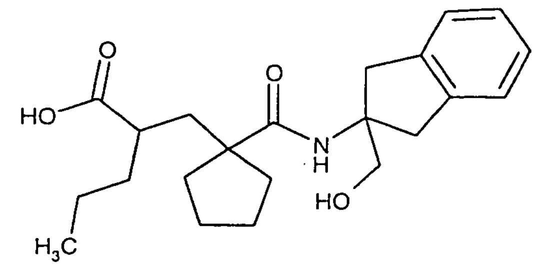
Cis-3-(2-Methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}cyclohexyl)-amino]carbonyl}cyclopentyl)methyl]propanoic acid (F61)



A solution of the *tert*-butyl ester from preparation 8 (8/66) (446mg, 0.75mmol) in dichloromethane (5ml) and trifluoroacetic acid (5ml) was stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and the residue azeotroped with dichloromethane, then toluene, and finally ether, to afford the title compound as a white foam, 385mg, 95%; ^{1}H NMR ($CDCl_3$, 400MHz) d: 1.48-2.17 (m, 18H), 2.40 (s, 1H), 2.66 (s, 1H), 3.37 (s, 3H), 3.50-3.70 (m, 6H), 3.94 (s, 1H), 6.10 (d, 1H), 6.59 (s, 1H), 7.55 (t, 2H), 7.61 (m, 1H), 8.02 (d, 2H), 9.11 (s, 1H); Anal. Found: C, 54.88; H, 6.90; N, 5.04. $C_{26}H_{38}N_2O_8S$;$1.7H_2O$ requires C, 57.97; H, 7.11; N, 5.20%.

Example 6

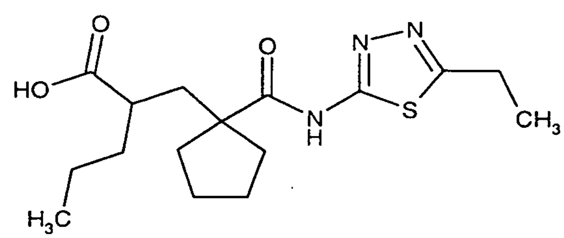
(+)-2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid (F62)



2-{[1-({[2-(Hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}pentanoic acid (WO 9110644) was further purified by HPLC using an AD column and hexane:isopropanol:trifluoroacetic acid (90:10:0.1) as eluant, to give the title compound of example 6, 99% ee, $[\alpha]_D = +10.4°$ (c = 0.067, ethanol).

Example 7

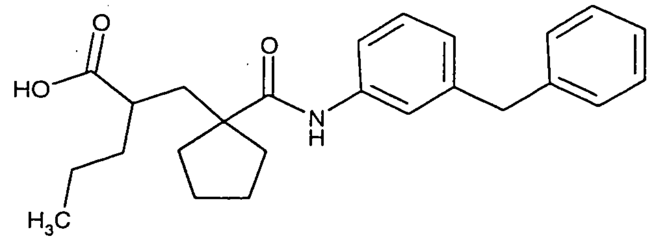
(+)-2-[(1-{[(5-Ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]pentanoic acid (F63)



The acid from Preparation 18 (18/ex4) (824mg) was further purified by HPLC using an AD column and using hexane:*iso*-propanol:trifluoroacetic acid (85:15:0.2) as eluant to give the title compound of example 7 as a white foam, 386mg, 99% ee, ^1H NMR ($CDCl_3$, 400MHz) δ: 0.90 (t, 3H), 1.38 (m, 6H), 1.50-1.79 (m, 9H), 2.19 (m, 1H), 2.30 (m, 1H), 2.44 (m, 1H), 2.60 (m, 1H), 2.98 (q, 2H), 12.10-12.27 (bs, 1H); LRMS: m/z 338 (MH^-); and $[\alpha]_D = +3.8°$ (c = 0.1, methanol)

Example 8

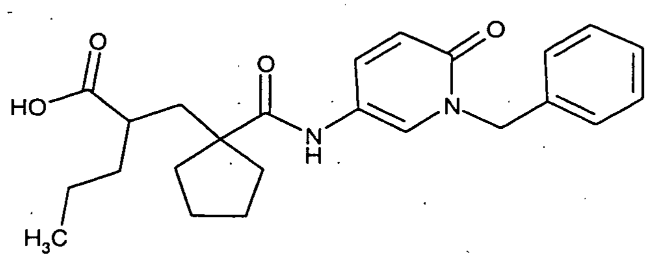
2-({1-[(3-Benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (F64).



A mixture of the benzyl ester from preparation 10 (10/53) (1.3mg, 2.47mmol) and 5% palladium on charcoal (130mg) in water (10ml) and ethanol (40ml) was hydrogenated at 30 psi and room temperature for 2 hours. The reaction mixture was fiiltered through Arbocel®, the filtrate concentrated under reduced pressure, and the residue triturated with dichloromethane. The residual gum was triturated with ether, then hexane, and dried at 50°C, to give the title compound as a solid, 0.79g, 81%; ^{1}H NMR ($CDCl_3$, 300MHz) d: 0.95 (t, 3H), 1.24-1.51 (m, 3H), 1.58-1.80 (m; 7H), 1.88 (dd, 1H), 2.15 (m, 2H), 2.24 (m, 1H), 2.48 (m, 1H), 4.00 (s, 2H), 6.98 (d, 1H), 7.24 (m, 6H), 7.40 (m, 3H); Anal. Found: C, 75.48; H, 7.76; N, 3.59. $C_{25}H_{31}NO_3$;$0.25H_2O$ requires C, 75.44; H, 7.98; N, 3.51%.

Example 9

2-[(1-{[(1-Benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]-pentanoic acid (F65)



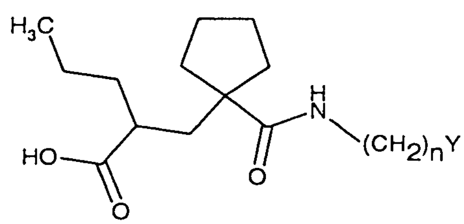
The title compound was obtained as a white foam in 51% yield from the benzyl ester from preparation 13 (13/56), following a similar procedure to that described in Preparation 19 (19/ex21), except, the product was purified by column chromatography on silica gel, using ethyl acetate as eluant; ^{1}H NMR ($CDCl_3$,

300MHz) d: 0.96 (t, 3H), 1.28-1.80 (m, 12H), 2.01 (m, 1H), 2.30-2.52 (m, 2H), 5.02 (dd, 2H), 6.60 (d, 1H), 7.27 (m, 5H), 7.70 (s, 1H), 8.34 (s, 1H); Anal. Found: C, 69.52; H, 7.41; N, 6.51. $C_{24}H_{30}N_2O_4$;$0.25H_2O$ requires C, 69.45; H, 7.41; N, 6.75.

Example 10

2-{[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (F66)

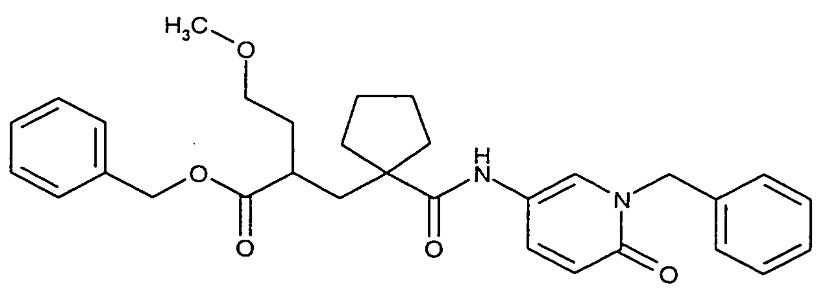
Compounds of formula ic, i.e. Compounds of general formula i where r^1 is propyl, where prepared from the corresponding *tert*-butyl ester, following a similar procedure to that described in Preparation 14 (14/ex1).



(Ic)

Preparation 1 (1/62)

Benzyl 2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)-methyl]-4-methoxybutanoate

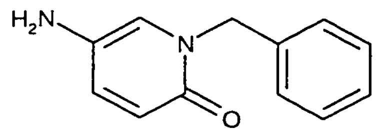


Oxalyl chloride (0.26ml, 3.0mmol) was added to an ice-cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-methoxybutyl}cyclopentanecarboxylic acid (EP 274234) (1.0g, 3.0mmol) and N,N-dimethylformamide (2 drops) in dichloromethane (20ml), and the reaction stirred at room temperature for 2 hours. The solution was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x10ml). The product was dissolved in dichloromethane (20ml), then cooled in an ice-bath. The amine from preparation 2 (2/28) (600mg, 3mmol) and N-methylmorpholine (0.6ml, 5.45mmol) were added and the reaction stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, and partitioned between water and ether. The organic layer was washed

with hydrochloric acid (2N), sodium bicarbonate solution, then water, dried ($MgSO_4$) and evaporated under reduced pressure. The residual green solid was purified by medium pressure column chromatography on silica gel using ethyl acetate:hexane (90:10) as eluant to afford the title compound, 880mg, 57%; ^{1}H NMR ($CDCl_3$, 300MHz) d: 1.37-2.28 (m, 12H), 2.46-2.64 (m, 1H), 3.20 (s, 3H), 3.31 (m, 2H), 4.97 (dd, 2H), 5.08 (dd, 2H), 6.57 (d, 1H), 7.12 (m, 1H), 7.18-7.48 (m, 10H), 8.08 (d, 1H).

Preparation 2 (2/28)

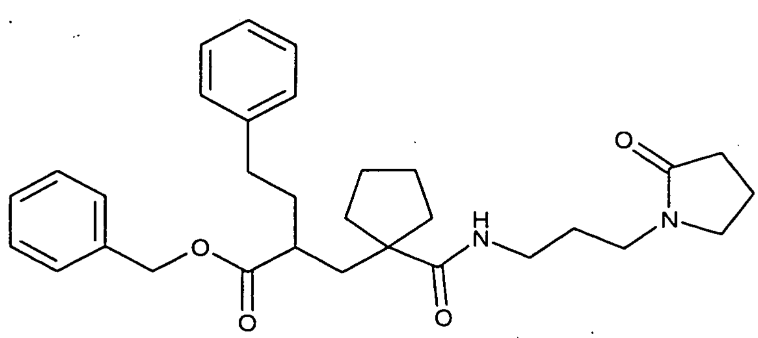
5-Amino-1-benzyl-2(1H)-pyridinone



A mixture of 1-benzyl-5-nitro-1H-pyridin-2-one (Justus Liebigs Ann. Chem. 484; 1930; 52) (1.0g, 4.35mmol), and granulated tin (3.5g, 29.5mmol) in concentrated hydrochloric acid (14ml) was heated at 90°C for 1.5 hours. The cooled solution was diluted with water, neutralised using sodium carbonate solution, and extracted with ethyl acetate (250ml in total). The combined organic extracts were filtered, dried ($MgSO_4$), and evaporated under reduced pressure to give the title compound as a pale green solid, (turned blue with time), 440mg, 51%; ^{1}H NMR ($CDCl_3$, 250MHz) δ: 4.12-4.47 (bs, 2H), 5.00 (s, 2H), 6.31 (d, 1H), 6.86 (s, 1H), 7.07 (m, 1H), 7.14-7.42 (m, 5H).

Preparation 3 (3/67)

Benzyl 2-{[1-({[3-(2-Oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoate

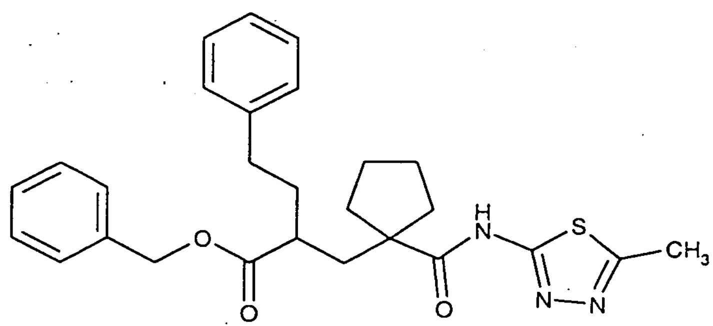


1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.06g, 5.53mmol), 1-hydroxybenzotriazole hydrate (0.60g, 4.44mmol) and 4-methylmorpholine (0.56g, 5.54mmol) were added sequentially to a cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (1.5g,

3.94mmol) in dry dichloromethane (15ml) at room temperature, followed by N-(3-aminopropyl)-2-pyrrolidinone (0.56g, 3.94mmol), and the reaction stirred at room temperature for 18 hours. The mixture was washed with water, 2N hydrochloric acid, saturated aqueous sodium bicarbonate solution, and then dried ($MgSO_4$) and evaporated under reduced pressure. The residual yellow oil was purified by column chromatography on silica gel using ethyl acetate:pentane (50:50) as the eluant to provide the title compound as a clear gum, 800mg, 40%; ^1H NMR ($CDCl_3$, 300MHz) d : 1.37-2.20 (m, 16H), 2.34-2.58 (m, 5H), 2.92-3.46 (m, 6H), 5.07 (d, 1H), 5.18 (d, 1H), 6.98-7.47 (m, 10H).

Preparation 4 (4/70)

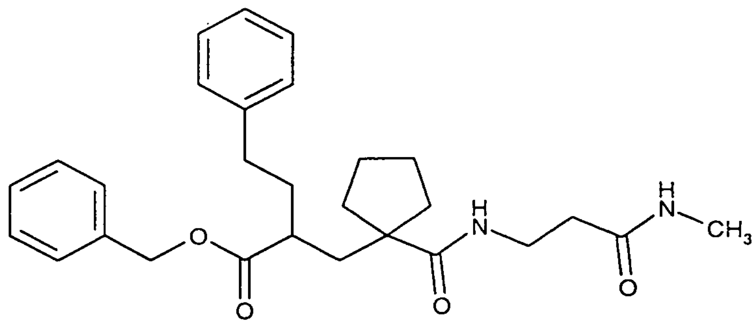
Benzyl 2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)methyl]-4-phenylbutanoate



The title compound was obtained as a clear oil in 74% yield from 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) and 2-amino-5-methyl-1,3,4-thiadiazole, following a similar procedure to that described in preparation 5 (5/68); ^1H NMR ($CDCl_3$, 400MHz) d: 1.58-1.76 (m, 7H), 1.83-1.98 (m, 3H), 2.03 (m, 1H), 2.20 (m, 1H), 2.35 (m, 1H), 2.44 (m, 3H), 2.65 (s, 3H), 5.02 (dd, 2H), 7.00 (d, 2H), 7.15 (m, 1H), 7.19 (m, 2H), 7.35 (m, 5H); LRMS : m/z 478.7 (MH^+).

Preparation 5 (5/68)

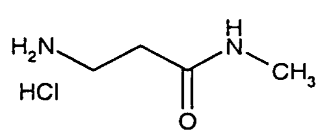
Benzyl 2-{[1-({[3-(methylamino)-3-oxopropyl]amino}carbonyl)cyclopentyl]methyl}-4-phenylbutanoate



1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (122mg, 0.64mmol), 1-hydroxybenzotriazole hydrate (86mg, 0.64mmol) and 4-methylmorpholine (173μl, 1.59mmol) were added sequentially to a cooled solution of 1-{2-[(benzyloxy)carbonyl]-4-phenylbutyl}cyclopentane-carboxylic acid (EP 274234) (202mg, 0.53mmol) in N,N-dimethylformamide (5ml) at room temperature, followed by the amine hydrochloride from preparation 6 (6/23) (146mg, 1.06mmol), and the reaction stirred at 90°C for 18 hours. The cooled solution was concentrated under reduced pressure and the residue partitioned between water (20ml) and ethyl acetate (100ml). The layers were separated, the organic phase washed with water (3x30ml), brine (25ml) dried ($MgSO_4$), and evaporated under reduced pressure to give a clear oil. The crude product was purified by column chromatography on silica gel using dichloromethane:methanol (98:2) as eluant to afford the title compound as a colourless oil, 162mg, 67%; ^{1}H NMR ($CDCl_3$, 400MHz) δ: 1.38-1.53 (m, 2H), 1.53-1.96 (m, 8H), 2.02 (m, 2H), 2.27 (t, 2H), 2.46 (m, 3H), 2.76 (d, 3H), 3.44 (m, 2H), 5.13 (s, 2H), 5.79 (bs, 1H), 6.38 (m, 1H), 7.06 (d, 2H), 7.18 (m, 1H), 7.22 (m, 2H), 7.38 (m, 5H); LRMS : m/z 465.5 (MH^+).

Preparation 6 (6/23)

3-Amino-N-methylpropanamide hydrochloride

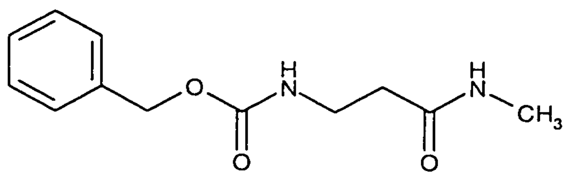


A mixture of the benzyl carbamate from preparation 7 (7/13) (7.92g, 33.5mmol) and 5% palladium on charcoal (800mg) in ethanol (300ml) was hydrogenated at 50 psi and room temperature for 4 hours. The reaction mixture was filtered through Arbocel

®, washing through with ethanol, and 1N hydrochloric acid (36.9ml, 36.9mmol) was added to the combined filtrate. This solution was evaporated under reduced pressure and the residue azeotroped with dichloromethane to afford the title compound as a colourless foam, 4.66g, [1]H NMR (DMSOd$_6$, 300MHz) δ: 2.46 (t, 2H), 2.60 (s, 3H), 2.95 (m, 2H), 7.98-8.16 (m, 2H).

Preparation 7 (7/13)

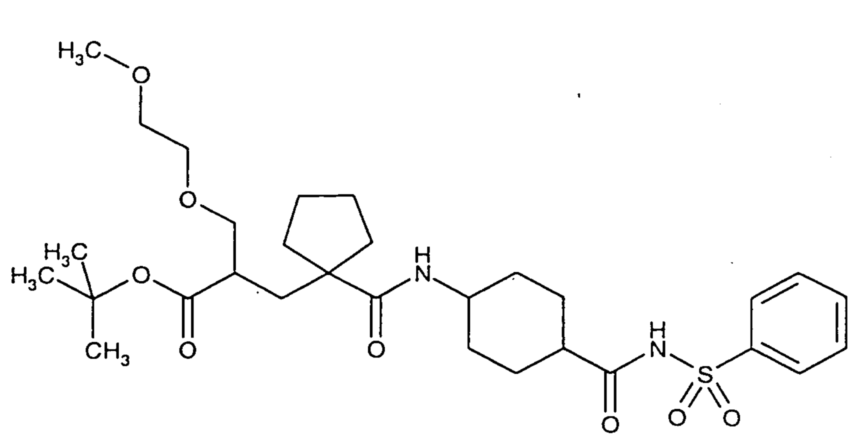
Benzyl 3-(methylamino)-3-oxopropylcarbamate



A mixture of N-[(benzyloxy)carbonyl]-β-alanine (10g, 44.8mmol), methylamine hydrochloride (3.33g, 49.28mmol), 1-hydroxybenzotriazole hydrate (6.05g, 44.8mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10.3g, 53.76mmol) and N-methylmorpholine (11.33ml, 103mmol) in dichloromethane (200ml) was stirred at room temperature for 18 hours. The resulting precipitate was filtered off to give the desired product as a colourless foam, and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using an elution gradient of ethyl acetate:hexane (90:10 to 100:0) to give additional product, 7.96g, 75% in total; [1]H NMR (CDCl$_3$, 300MHz) δ: 2.42 (t, 2H), 2.80 (s, 3H), 3.50 (m, 2H), 5.21 (s, 2H), 5.49 (bs, 1H), 5.63 (bs, 1H), 7.36 (m, 5H); Anal. Found: C, 60.68; H, 7.00; N, 11.95. $C_{12}H_{16}N_2O_3$ requires C, 61.00; H, 6.83; N, 11.86%.

Preparation 8 (8/66)

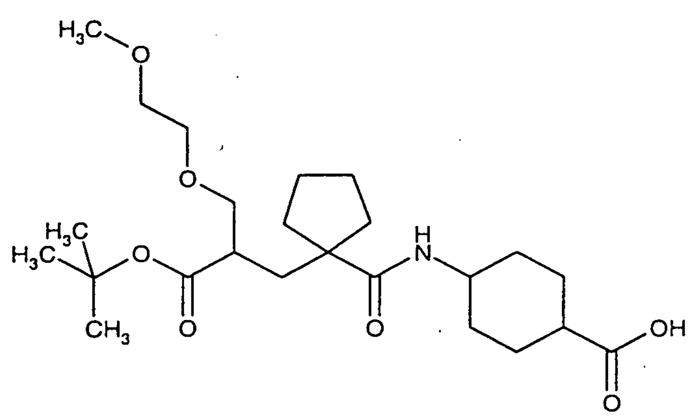
Cis-tert-Butyl 3-(2-methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}-cyclohexyl)amino]carbonyl}cyclopentyl)methyl]propanoate



N,N'-Dicyclohexylcarbodiimide (199mg, 0.97mmol), 4-dimethylaminopyridine (118mg, 0.97mmol) and benzenesulphonamide (152mg, 0.97mmol) were added to an ice-cooled solution of the acid from preparation 9 (9/63) (400mg, 0.878mmol) in dichloromethane (12ml) and N,N-dimethylformamide (0.5ml), and the reaction stirred at room temperature for 20 hours. The mixture was concentrated under reduced pressure and the residue suspended in cold ethyl acetate. The resulting insoluble material was filtered off, the filtrate washed with hydrochloric acid (1N), and water, then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using an elution gradient of dichloromethane:methanol (95:5 to 90:10) to afford the title compound as a white foam, 480mg, 92%; ^{1}H NMR ($CDCl_3$, 400MHz) d: 1.44 (s, 9H), 1.63 (m, 13H), 1.80 (m, 2H), 1.88 (m, 1H), 1.98 (m, 2H), 2.36 (m, 1H), 2.57 (m, 1H), 3.38 (s, 3H), 3.40 (m, 1H), 3.51 (t, 2H), 3.58 (m, 3H), 3.95 (m, 1H), 5.92 (d, 1H), 7.56 (m, 2H), 7.62 (m, 1H), 8.05 (d, 2H), 8.75 (bs, 1H); LRMS : m/z 618 (MNa^+).

Preparation 9 (9/63)

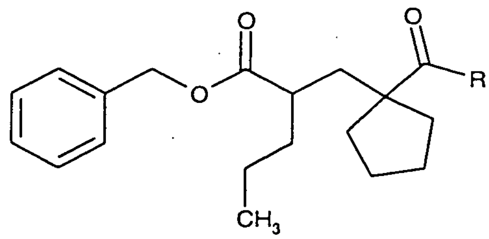
4-{[(1-{3-tert-Butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)-carbonyl]amino}cyclohexanecarboxylic acid



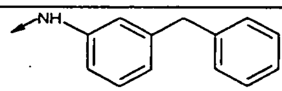
A mixture of benzyl 4-{[(1-{3-tert-butoxy-2-[(2-methoxyethoxy)methyl]-3-oxopropyl}cyclopentyl)carbonyl]amino}cyclohexanecarboxylate (EP 274234), and 10% palladium on charcoal (250mg) in water (10ml) and ethanol (50ml) was hydrogenated at 50 psi and room temperature for 18 hours. The reaction mixture was filtered through Solkafloc®, the filtrate concentrated under reduced pressure and the residue azeotroped with toluene (3x) and then dichloromethane (3x), to give the title compound, 2.0g, 96%; ^{1}H NMR ($CDCl_3$, 300MHz) d: 1.48 (s, 9H), 1.53-1.84 (m, 14H), 1.94-2.10 (m, 5H), 2.60 (m, 2H), 3.40 (s, 3H), 3.41-3.63 (m, 5H), 3.96 (m, 1H), 5.90 (bd, 1H).

Preparation 10 (10/53)

The following compound:



where:

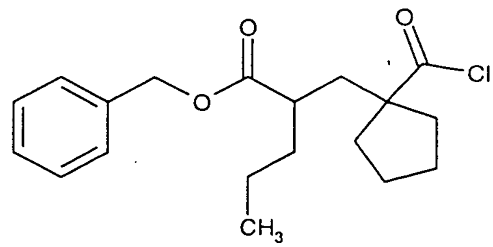
Prep	R	Yield (%)	Data
10 (10/53)[1]		90	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.24 (m, 2H), 1.40-1.76 (m, 7H), 1.84 (dd, 1H), 1.98 (m, 1H), 2.19 (dd, 1H), 2.28 (m, 1H), 2.56 (m, 1H), 3.98 (s, 2H), 4.99 (dd, 2H), 6.98 (d, 1H), 7.19-7.42 (m, 15H).

1 = dichloromethane used as the column eluant

was prepared from the acid chloride from preparation 11 (11/3) and the appropriate amine, following a similar procedure to that described in preparation 12 (12/52).

Preparation 11 (11/3)

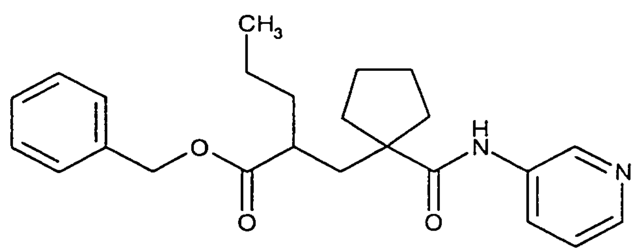
Benzyl 2-{[1-(chlorocarbonyl)cyclopentyl]methyl}pentanoate



Oxalyl chloride (1.15ml, 13.2mmol) was added to an ice-cooled solution of 1-{2-[(benzyloxy)carbonyl]pentyl}cyclopentanecarboxylic acid (EP 274234) (2.0g, 6.3mmol) in dry dichloromethane (20ml), and the solution stirred at room temperature for 2 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with dichloromethane (3x), to give the title compound as a golden oil, 2.1g; ^{1}H NMR ($CDCl_3$, 300MHz) d: 0.88 (t, 3H), 1.28 (m, 2H), 1.43 (m, 2H), 1.63 (m, 6H), 2.00 (m, 1H), 2.08-2.35 (m, 3H), 2.44 (m, 1H), 5.15 (s, 2H), 7.28 (m, 5H).

Preparation 12 (12/52)

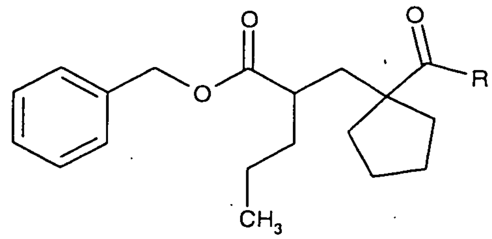
Benzyl 2-({1-[(3-pyridinylamino)carbonyl]cyclopentyl}methyl)pentanoate



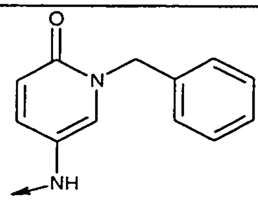
Triethylamine (0.11ml, 0.78mmol) was added to a mixture of the acid chloride from preparation 11 (11/3) (200mg, 0.60mmol) and 2-aminopyridine (61mg, 0.65mmol) in dichloromethane (3ml), and the reaction stirred at room temperature for 16 hours. The mixture was evaporated under reduced pressure, the residue partitioned between sodium bicarbonate solution (5ml) and ethyl acetate (20ml), and the layers separated. The organic phase was dried ($MgSO_4$), and evaporated under reduced pressure to give a gum. The crude product was purified by column chromatography on silica gel using ethyl acetate as eluant, to afford the title compound, 130mg; ^{1}H NMR ($CDCl_3$, 400MHz) d: 0.82 (t, 3H), 1.21 (m, 3H), 1.40 (m, 1H), 1.43-1.72 (m, 6H), 1.81 (d, 1H), 1.98 (m, 1H), 2.18 (m, 1H), 2.24 (m, 1H), 2.46 (m, 1H), 4.98 (m, 2H), 7.20-7.38 (m, 6H), 7.42 (s, 1H), 8.06 (d, 1H), 8.35 (d, 1H), 8.56 (s, 1H).

Preparation 13 (13/56)

The following compound:



where:

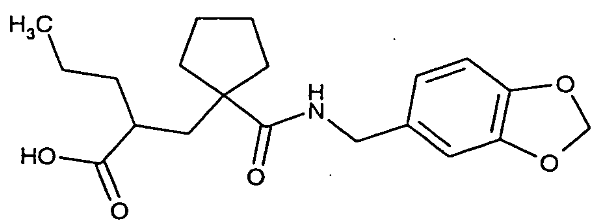
Prep	R	Yield (%)	Data
13 (13/56)[2]		53	^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.84 (t, 3H), 1.25 (m, 2H), 1.27-1.99 (m, 10H), 2.07-2.30 (m, 2H), 2.47 (m, 1H), 4.99 (s, 2H), 5.10 (dd, 2H), 6.59 (d, 1H), 7.15 (d, 1H), 7.34 (m, 11H), 8.10 (s, 1H).

2 = N-methylmorpholine was used as the base

was prepared from the acid chloride from preparation 11 (11/3) and the appropriate amine, following a similar procedure to that described in preparation 12 (12/52).

Preparation 14 (14/ex 1)

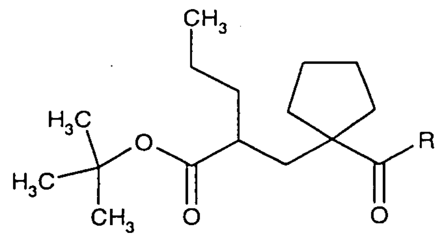
2-({1-[(1,3-Benzodioxol-5-ylamino)carbonyl]cyclopentyl}methyl)pentanoic acid



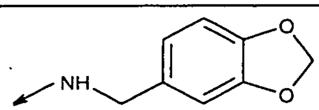
Trifluoroacetic acid (5ml) was added to a solution of the tert-butyl ester from preparation 15 (15/34) (130mg, 0.31mmol) in dichloromethane (5ml), and the solution stirred at room temperature for 4 hours. The reaction mixture was concentrated under reduced pressure and the residue azeotroped with toluene and dichloromethane to afford the title compound as a clear oil, 112 mg, ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.83 (t, 3H), 1.22-1.40 (m, 3H), 1.50-1.72 (m, 8H), 1.95 (m, 1H), 2.10 (m, 2H), 2.19 (m, 1H), 4.30 (m, 2H), 5.93 (s, 2H), 5.99 (bs, 1H), 6.74 (m, 3H); LRMS: m/z 380 (MH^-).

Preparation 15 (15/34)

The following compound:



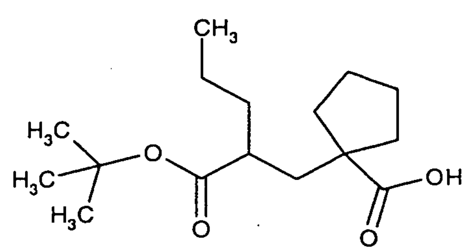
where

Prep	R	Starting amine	Yield (%)	Data
15 (15/34)		Piperonylamine	88	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.85 (t, 3H), 1.26 (m, 4H), 1.42 (s, 9H), 1.46 (m, 2H), 1.59-1.75 (m, 5H), 1.95 (m, 2H), 2.06 (m, 1H), 2.22 (m, 1H), 4.26 (dd, 1H), 4.39 (dd, 1H), 5.95 (m, 3H), 6.78 (m, 3H). LRMS : m/z 418.3 (MH^+)

was prepared from the acid from preparation 16 (16/1) and the appropriate amine compound, following a similar procedure to that described in preparation 17 (17/33).

Preparation 16 (16/1)

1-[2-(tert-Butoxycarbonyl)-4-pentyl]-cyclopentane carboxylic acid

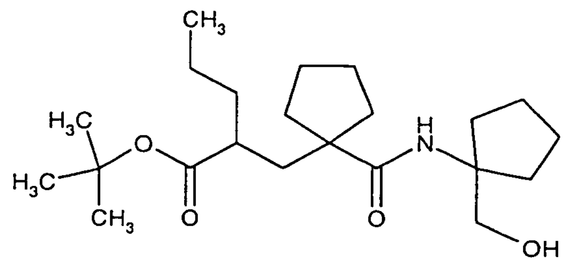


A mixture of 1-[2-(tert-butoxycarbonyl)-4-pentenyl]-cyclopentane carboxylic acid (EP 274234) (23g, 81.5mmol) and 10% palladium on charcoal (2g) in dry ethanol (200ml) was hydrogenated at 30psi and room temperature for 18 hours. The reaction mixture was filtered through Arbocel®, and the filtrate evaporated under reduced pressure to give a yellow oil. The crude product was purified by column chromatography on silica

gel, using ethyl acetate:pentane (40:60) as the eluant, to provide the desired product as a clear oil, 21g, 91%; ^{1}H NMR ($CDCl_3$, 0.86 (t, 3H), 1.22-1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd, 1H), 2.00-2.18 (m, 3H), 2.24 (m, 1H); LRMS : m/z 283 $(M-H)^-$

Preparation 17 (17/33)

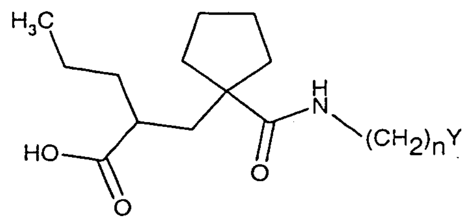
tert-Butyl 2-{[1-({[1-(hydroxymethyl)cyclopentyl]amino}carbonyl)-cyclopentyl]methyl}pentanoate



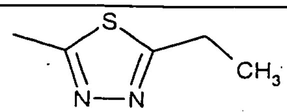
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (41mg, 0.21mmol), 1-hydroxybenzotriazole hydrate (27mg, 0.2mmol), N-methylmorpholine (35μl, 0.31mmol) and finally 1-amino-1-cyclopentanemethanol (25mg, 0.22mmol) were added to a solution of the acid from preparation 16 (16/1) (150mg, 0.53mmol) in N,N-dimethylformamide (3ml), and the reaction stirred at 90°C for 18 hours. The cooled solution was diluted with ethyl acetate (90ml), washed with water (3x25ml), and brine (25ml), then dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using ethyl acetate:pentane (30:70) as the eluant to afford the title compound, 38mg, 57%; ^{1}H NMR ($CDCl_3$, 400MHz) d: 0.88 (t, 3H), 1.29 (m, 3H), 1.41-1.78 (m, 26H), 1.78-1.98 (m, 4H), 2.04 (m, 1H), 2.26 (m, 1H), 3.59 (dd, 1H), 3.70 (dd, 1H), 4.80 (t, 1H), 5.81 (s, 1H); LRMS : m/z 380 (MH^-).

Preparation 18 (18/ex.4)

A compound of the formula shown below was prepared from the corresponding *tert*-butyl ester following a similar procedure to that described in Preparation 14 (14/ex.1).



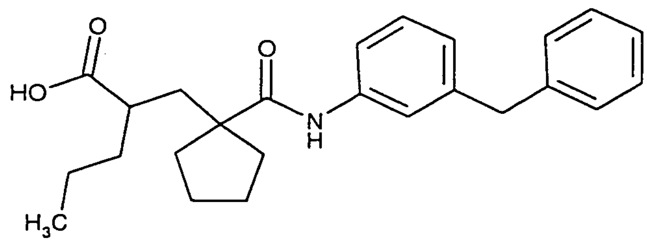
(Ic)

Ex	N	R	Yield	Data
18 (18/ ex.4)[3]	0		86	^{1}H NMR ($CDCl_3$, 400MHz) δ: 0.92 (t, 3H), 1.35 (t, 3H), 1.25-1.80 (m, 11H), 2.20-2.50 (m, 4H), 2.95 (q, 2H), 12.10 (bs, 1H). LRMS : m/z 339.8 (MH^+) Anal. Found: C, 56.46; H, 7.46; N, 12.36. $C_{16}H_{25}N_3O_3S$ requires C, 56.62; H, 7.44; N, 12.37%.

3 = recrystallised from ether

Preparation 19 (19/ex.21)

2-({1-[(3-Benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid



A mixture of the benzyl ester from preparation 10 (10/53) (1.3mg, 2.47mmol) and 5% palladium on charcoal (130mg) in water (10ml) and ethanol (40ml) was hydrogenated at 30 psi and room temperature for 2 hours. The reaction mixture was fiiltered through Arbocel®, the filtrate concentrated under reduced pressure, and the residue triturated with dichloromethane. The residual gum was triturated with ether, then hexane, and dried at 50°C, to give the title compound as a solid, 0.79g, 81%; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.95 (t, 3H), 1.24-1.51 (m, 3H), 1.58-1.80 (m, 7H), 1.88 (dd, 1H), 2.15 (m, 2H), 2.24 (m, 1H), 2.48 (m, 1H), 4.00 (s, 2H), 6.98 (d, 1H), 7.24 (m, 6H), 7.40 (m, 3H); Anal. Found: C, 75.48; H, 7.76; N, 3.59. $C_{25}H_{31}NO_3$;$0.25H_2O$ requires C, 75.44; H, 7.98; N, 3.51%.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

REFERENCES FOR THE NEP SECTION

1. Barker, P. E.; Shipp, M. A.; D'Adamio, L.; Masteller, E. L.; Reinherz, E. L. The common acute lymphoblastic leukemia antigen gene maps to chromosomal region 3(q21-q27). J. Immun. 142: 283-287, 1989.

2. D'Adamio, L.; Shipp, M. A.; Masteller, E. L.; Reinherz, E. L. : Organization of the gene encoding common acute lymphoblastic leukemia antigen (neutral endopeptidase 24.11): multiple miniexons and separate 5-prime untranslated regions. Proc. Nat. Acad. Sci. 86: 7103-7107, 1989.

3. Letarte, M.; Vera, S.; Tran, R.; Addis, J. B. L.; Onizuka, R. J.; Quackenbush, E. J.; Jongeneel, C. V.; McInnes, R. R. : Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. J. Exp. Med. 168: 1247-1253, 1988.

4. Shipp, M. A.; Vijayaraghavan, J.; Schmidt, E. V.; Masteller, E. L.; D'Adamio, L.; Hersh, L. B.; Reinherz, E. L. : Common acute lymphoblastic leukemia antigen (CALLA) is active neutral endopeptidase 24.11 ('enkephalinase'): direct evidence by cDNA transfection analysis. Proc. Nat. Acad. Sci. 86: 297-301, 1989.

5. Tran-Paterson, R.; Willard, H. F.; Letarte, M. : The common acute lymphoblastic leukemia antigen (neutral endopeptidase--3.4.24.11) gene is located on human chromosome 3. Cancer Genet. Cytogenet. 42: 129-134, 1989.

REFERENCES FOR THE NPY SECTION

1. Allen, J. M.; Bloom, S. R. : Neuropeptide Y: a putative neurotransmitter. Neurochem. Int. 8: 1-8, 1986.

2. Bahary, N.; Zorich, G.; Pachter, J. E.; Leibel, R. L.; Friedman, J. M. : Molecular genetic linkage maps of mouse chromosomes 4 and 6. Genomics 11: 33-47, 1991.

3. Baker, E.; Hort, Y. J.; Ball, H.; Sutherland, G. R.; Shine, J.; Herzog, H. : Assignment of the human neuropeptide Y gene to chromosome 7p15.1 by nonisotopic in situ hybridization. Genomics 26: 163-164, 1995.

1. Carr, L. G.; Foroud, T.; Bice, P.; Gobbett, T.; Ivashina, J.; Edenberg, H.; Lumeng, L.; Li, T. K. : A quantitative trait locus for alcohol consumption in selectively bred rat lines. Alcohol Clin. Exp. Res. 22: 884-887, 1998.

5. Dockray, G. J. : Neuropeptide Y: in search of a function. Neurochem. Int. 8: 9-11, 1986.

6. Erickson, J. C.; Clegg, K. E.; Palmiter, R. D. : Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature 381: 415-421, 1996. PubMed ID : 8632796

7. Erickson, J. C.; Hollopeter, G.; Palmiter, R. D. : Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. Science 274: 1704-1706, 1996.

8. Karvonen, M. K.; Pesonen, U.; Koulu, M.; Niskanen, L.; Laakso, M.; Rissanen, A.; Dekker, J. M.; 't Hart, L. M.; Valve, R.; Uusitupa, M. I. : Association of a leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y with high serum cholesterol and LDL cholesterol levels. Nature Med. 4: 1434-1437, 1998.

9. Maccarrone, C.; Jarrott, B. : Neuropeptide Y: a putative neurotransmitter. Neurochem. Int. 8: 13-22, 1986.

10. Meisler, M. H.; Spence, J. E.; Dixon, J. E.; Caldwell, R. M.; Minth, C. D.; Beaudet, A. L. : Exclusion of close linkage between the loci for cystic fibrosis and neuropeptide Y on human chromosome 7. Cytogenet. Cell Genet. 44: 175-176, 1987.

11. Minth, C. D.; Andrews, P. C.; Dixon, J. E. : Characterization, sequence, and expression of the cloned human neuropeptide Y gene. J. Biol. Chem. 261: 11974-11979, 1986.

12. Minth, C. D.; Bloom, S. R.; Polak, J. M.; Dixon, J. E. : Cloning, characterization, and DNA sequence of a human cDNA encoding neuropeptide tyrosine. Proc. Nat. Acad. Sci. 81: 4577-4581, 1984.

13. Takeuchi, T.; Gumucio, D.; Eddy, R.; Meisler, M.; Minth, C.; Dixon, J.; Yamada, T.; Shows, T. : Assignment of the related pancreatic polypeptide (PPY) and neuropeptide Y (NPY) genes to regions on human chromosomes 17 and 7. (Abstract) Cytogenet. Cell Genet. 40: 759 only, 1985.

14. Takeuchi, T.; Gumucio, D. L.; Yamada, T.; Meisler, M. H.; Minth, C. D.; Dixon, J. E.; Eddy, R. E.; Shows, T. B. : Genes encoding pancreatic polypeptide and neuropeptide Y are on human chromosomes 17 and 7. J. Clin. Invest. 77: 1038-1041, 1986.

15. Terenghi, G.; Polak, J. M.; Hamid, Q.; O'Brien, E.; Denny, P.; Legon, S.; Dixon, J.; Minth, C. D.; Palay, S. L.; Yasargil, G.; Chan-Palay, V. : Localization of neuropeptide Y mRNA in neurons of human cerebral cortex by means of in situ hybridization with a complementary RNA probe. Proc. Nat. Acad. Sci. 84: 7315-7318, 1987.

16. Thiele, T. E.; Marsh, D. J.; Ste. Marie, L.; Bernstein, I. L.; Palmiter, R. D. : Ethanol consumption and resistance are inversely related to neuropeptide Y levels. Nature 396: 366-369, 1998.

17. Uusitupa, M. I. J.; Karvonen, M. K.; Pesonen, U.; Koulu, M. : Neuropeptide Y: a novel link between the neuroendocrine system and cholesterol metabolism. Ann. Med. 30: 508-510, 1998.

REFERENCES FOR THE NPYR1 SECTION

1. Herzog, H.; Baumgartner, M.; Vivero, C.; Selbie, L. A.; Auer, B.; Shine, J. : Genomic organization, localization, and allelic differences in the gene for the human neuropeptide Y Y1 receptor. J. Biol. Chem. 268: 6703-6707, 1993.

2. Herzog, H.; Darby, K.; Ball, H.; Hort, Y.; Beck-Sickinger, A.; Shine, J. : Overlapping gene structure of the human neuropeptide Y receptor subtypes Y1 and Y5 suggests coordinate transcriptional regulation. Genomics 41: 315-319, 1997.

3. Herzog, H.; Hort, Y. J.; Ball, H. J.; Hayes, G.; Shine, J.; Selbie, L. A. : Cloned human neuropeptide Y receptor couples to two different second messenger systems. Proc. Nat. Acad. Sci. 89: 5794-5798, 1992.

4. Larhammar, D.; Blomqvist, A. G.; Yee, F.; Jazin, E.; Yoo, H.; Wahlestedt, C. : Cloning and functional expression of a human neuropeptide Y/peptide YY receptor of the Y1 type. J. Biol. Chem. 267: 10935-10938, 1992.

5. Lutz, C. M.; Frankel, W. N.; Richards, J. E.; Thompson, D. A. : Neuropeptide Y receptor genes on human chromosome 4q31-q32 map to conserved linkage groups on mouse chromosomes 3 and 8. Genomics 41: 498-500, 1997.

ABBREVIATIONS

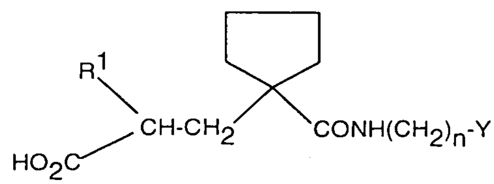
cAMP	=	cyclic adenosine-3',5'-monophosphate
cGMP	=	cyclic guanosine-3',5'-monophosphate
P_{cGMP}	=	potentiator of cGMP
NEP	=	neutral endopeptidase
NEPi	=	inhibitor of NEP (also known as I:NEP)
VIP	=	vasoactive intestinal peptide
PDE	=	phosphodiesterase

PDEn	=	PDE family (e.g. PDE1, PDE2 etc.)
PDE_{cGMP}	=	cGMP hydrolysing PDE
PDEi	=	inhibitor of a PDE (also known as I:PDE)
NPY	=	neuropeptide Y
I:NPY	=	inhibitor of NPY
kDa	=	kilodalton
bp	=	base pair
kb	=	kilobase pair

Claims

1 The use of a NEPi compound for the treatment of MED.

2 Use according to claim 1 wherein the NEPi is a compound of formula I (or a pharmaceutically acceptable salt, solvate or prodrug thereof):



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6}alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}$alkyl); or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

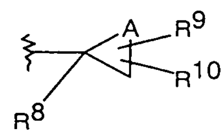
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or $-NR^2R^3$ wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

the -(CH_2)$_n$- linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

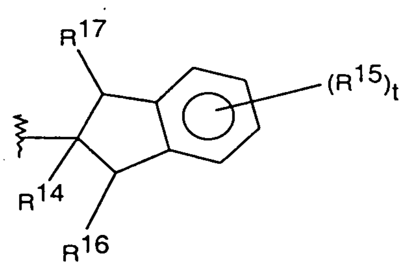
Y is the group



wherein A is -(CH_2)$_q$- where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, -CH_2OH, phenyl, phenyl(C_{1-4}alkyl) or CONR^{11}R^{12}; R^9 and R^{10} are each independently H, -CH_2OH, -C(O)NR^{11}R^{12}, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl, or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or S(O)$_r$R^{13}, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

Y is the group, -C(O) NR^{11} R^{12} wherein R^{11} and R^{12} are as previously defined except that R^{11} and R^{12} are not both H; or

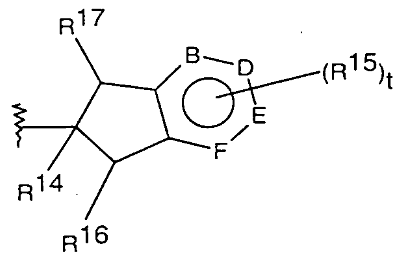
Y is the group,



wherein R^{14} is H, CH_2OH, or C(O)NR^{11}R^{12} wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t

is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

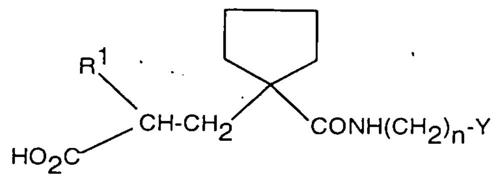
C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl; and

u is 0, 1, 2 or 3.

3 Use according to claim 1 wherein the NEPi is compound of formula (I), or a pharmaceutically acceptable salt, solvate or prodrug thereof:



(I)

wherein

R^1 is C_{1-6}alkyl which may be substituted by one or more substituents, which may be the same or different, selected from the list: halo, hydroxy, C_{1-6} alkoxy, C_{2-6} hydroxyalkoxy, C_{1-6}alkoxy(C_{1-6}alkoxy), C_{3-7}cycloalkyl, C_{3-7}cycloalkenyl, aryl, aryloxy, (C_{1-4}alkoxy)aryloxy, heterocyclyl, heterocyclyloxy, $-NR^2R^3$, $-NR^4COR^5$, $-NR^4SO_2R^5$, $-CONR^2R^3$, $-S(O)_pR^6$, $-COR^7$ and $-CO_2(C_{1-4}alkyl)$; or R^1 is C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more substituents from said list, which substituents may be the same or different, which list further includes C_{1-6}alkyl; or R^1 is C_{1-6} alkoxy, $-NR^2R^3$ or $-NR^4SO_2R^5$;

wherein

R^2 and R^3 are each independently H, C_{1-4}alkyl, C_{3-7}cycloalkyl (optionally substituted by hydroxy or C_{1-4}alkoxy), aryl, (C_{1-4}alkyl)aryl, C_{1-6}alkoxyaryl or heterocyclyl; or R^2 and R^3 together with the nitrogen to which they are attached form a pyrrolidinyl, piperidino, morpholino, piperazinyl or *N*-(C_{1-4} alkyl)piperazinyl group;

R^4 is H or C_{1-4}alkyl;

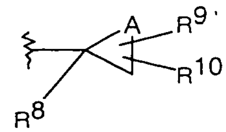
R^5 is C_{1-4}alkyl, CF_3, aryl, (C_{1-4} alkyl)aryl, (C_{1-4}alkoxy)aryl, heterocyclyl, C_{1-4}alkoxy or $-NR^2R^3$ wherein R^2 and R^3 are as previously defined;

R^6 is C_{1-4}alkyl, aryl, heterocyclyl or NR^2R^3 wherein R^2 and R^3 are as previously defined; and

R^7 is C_{1-4}alkyl, C_{3-7}cycloalkyl, aryl or heterocyclyl; n is 0, 1 or 2; p is 0, 1, 2 or 3;

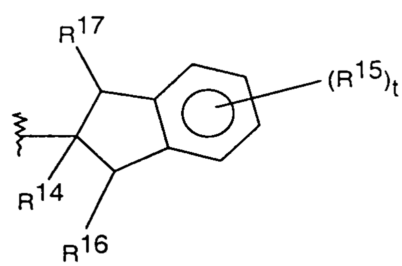
the $-(CH_2)_n-$ linkage is optionally substituted by C_{1-4}alkyl, C_{1-4}alkyl substituted with one or more fluoro groups or phenyl, C_{1-4}alkoxy, hydroxy, hydroxy(C_{1-3}alkyl), C_{3-7}cycloalkyl, aryl or heterocyclyl;

Y is the group



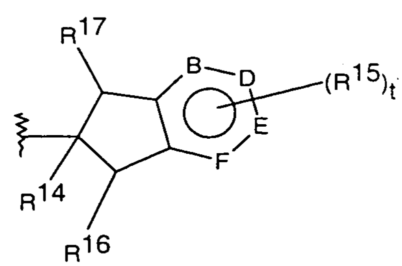
wherein A is $-(CH_2)_q-$ where q is 1, 2, 3 or 4 to complete a 3 to 7 membered carbocyclic ring which may be saturated or unsaturated; R^8 is H, C_{1-6}alkyl, $-CH_2OH$, phenyl, phenyl(C_{1-4}alkyl) or $CONR^{11}R^{12}$; R^9 and R^{10} are each independently H, $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl, or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl, or R^9 and R^{10} together form a dioxolane; $R^{11}$and R^{12} which may be the same or different are H, C_{1-4}alkyl, R^{13} or $S(O)_rR^{13}$, where r is 0, 1 or 2 and R^{13} is phenyl optionally substituted by C_{1-4}alkyl or phenylC_{1-4}alkyl wherein the phenyl is optionally substituted by C_{1-4}alkyl; or

Y is the group,



wherein R^{14} is $C(O)NR^{11}R^{12}$ wherein R^{11} and R^{12} are as previously defined; when present R^{15}, which may be the same or different to any other R^{15}, is OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; t is 0, 1, 2, 3 or 4; and R^{16} and R^{17} are independently H or C_{1-4} alkyl; or

Y is the group



wherein one or two of B, D, E or F is a nitrogen, the others being carbon; and R^{14} to R^{17} and t are as previously defined; or

Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may be optionally benzofused and optionally substituted by:

C_{1-6} alkoxy; hydroxy; oxo; amino; mono or di-(C_{1-4}alkyl)amino; C_{1-4}alkanoylamino; or

C_{1-6}alkyl which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl; or

C_{3-7}cycloalkyl, aryl or heterocyclyl, each of which may be substituted by one or more groups, which may be the same or different, selected from the list: C_{1-6}alkyl, C_{1-6}alkoxy, C_{1-6}haloalkoxy, C_{1-6}alkylthio, halogen, C_{3-7}cycloalkyl, heterocyclyl or phenyl;

wherein when there is an oxo substitution on the heterocyclic ring, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring; or

Y is $-NR^{18}S(O)_uR^{19}$, wherein R^{18} is H or C_{1-4}alkyl; R^{19} is aryl, arylC_{1-4}alkyl or heterocyclyl; and

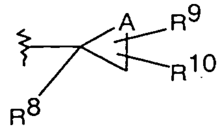
u is 0, 1, 2 or 3.

4. Use according to claim 1 wherein the NEPi is a compound as defined in claims 2 or 3 wherein R^1 is C_{1-6}alkyl, C_{1-6}alkoxy or C_{1-6}alkyl substituted with aryl.

5 Use of a compound as defined in claim 4 wherein R^1 is C_{1-6}alkyl, C_{1-6}alkoxy or C_{1-6}alkoxy(C_{1-3})alkyl.

6 Use of a compound as defined in claim 5 wherein R^1 is C_{1-4}alkyl.

7 Use of a compound as defined in any one of the preceding claims wherein when Y is the group



and the carbocyclic ring is fully saturated, then one of R^9 or R^{10} is $-CH_2OH$, $-C(O)NR^{11}R^{12}$, C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl.

8 Use of a compound as defined in claim 7 wherein the carbocyclic ring is 5, 6 or 7 membered wherein one of R^9 or R^{10}, $-C(O)NR^{11}R^{12}$, with the other being C_{1-6}alkyl, phenyl optionally substituted by C_{1-4}alkyl or phenyl(C_{1-4}alkyl) wherein the phenyl group is optionally substituted by C_{1-4}alkyl.

9 Use of a compound as defined in claim 8 wherein R^9 and R^{10} are attached to adjacent carbon atoms in the ring.

10 Use of a compound as defined in any one of the preceding claims wherein R^8 is CH_2OH.

11 Use of a compound as defined in any of claims 2 to 6 wherein when Y is the group $-NR^{18}S(O)_uR^{19}$, then R^{18} is H.

12 Use of a compound as defined in any of claims 2 to 6 or 11 wherein R^{19} is benzyl or phenyl.

13 Use of a compound as defined in any of claims 2 to 6 , 11 or 12 wherein u is 2.

14 Use of a compound as defined in claim 2 wherein Y is an optionally substituted 5-7 membered heterocyclic ring, which may be saturated, unsaturated or aromatic and contains a nitrogen, oxygen or sulphur and optionally one, two or three further nitrogen atoms in the ring and which may

be optionally benzofused and optionally substituted by one or more of C_{1-6} alkyl, phenyl, phenylC_{1-4}alkyl, C_{1-6} alkoxy, hydroxy, oxo, amino, mono or di-(C_{1-4}alkyl)amino or C_{1-4} alkanoylamino; with the proviso that when there is an oxo substitution, the ring only contains one or two nitrogen atoms and the oxo substitution is adjacent a nitrogen atom in the ring.

15 Use of a compound as defined in claim 14 wherein the heterocyclic ring is a heteroaromatic ring.

16 Use of a compound as defined in claim 15 wherein the heteroaromatic ring is selected from pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrazolyl, triazolyl, tetrazolyl, oxadiazole, thiazole, thiaiazole, oxazolyl, isoxazolyl, indolyl, isoindolinyl, quinolyl, pyridone, quinoxalinyl, and quinazolinyl each being optionally substituted as defined in claim 1.

17 Use of a compound as defined in claim 16 wherein the heteroaromatic ring is selected from oxadiazole, pyridone and thiadiazole.

18 Use of a compound as defined in claim 17 wherein the heteroaromatic ring is selected from 1,2,5 oxadiazole, 1,3,4 oxadiazole, 2-pyridone and 1,3,4 thiadiazole.

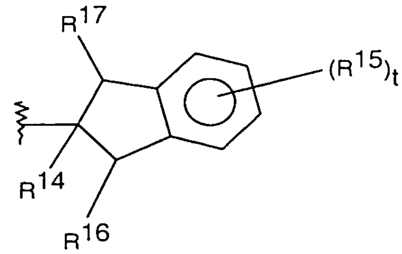
19 Use of a compound as defined in any of claims 14 to 18 wherein the heterocyclic ring is substituted by one or more C_{1-6}alkyl, phenyl or phenylC_{1-4}alkyl.

20 Use of a compound as defined in claim 19 wherein the substitution is C_{1-4}alkyl or benzyl.

21 Use of a compound as defined in claims 17 to 20 wherein when Y is a pyridone, said pyridone is *N*-substituted (preferably with benzyl or C_{1-4}alkyl).

22 Use of a compound as defined in claim 14 wherein Y is a lactam linked at the nitrogen.

23 Use of a compound as defined in claim 2 and claims dependent thereon wherein Y is



wherein R^{14} is H, CH_2OH or $C(O)NR^{11}R^{12}$, R^{15} is one or more of H, OH, C_{1-4}alkyl, C_{1-4}alkoxy, halo or CF_3; and R^{16} and R^{17} are independently H or C_{1-4}alkyl.

24 Use of a compound as defined in claim 23 wherein R^{14} is CH_2OH or $C(O)NR^{11}R^{12}$.

25 Use of a compound as defined in claim 24 wherein R^{14} is $C(O)NR^{11}R^{12}$.

26 Use of a compound as defined in claims 3 and claims 23 to 25 wherein R^{16} and R^{17} are H.

27 Use of a compound as defined in claims 3 and claims 22 to 25 wherein t is 0.

28 Use of a compound according to any one of the preceding claims selected from the group consisting of:

2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}-cyclopentyl)methyl]-4-methoxybutanoic acid (Example 35),

2-{[1-({[3-(2-oxo-1-pyrrolidinyl)propyl]amino}carbonylcyclopentyl]-methyl}-4-phenylbutanoic acid (Example 40),

(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)cyclopentyl]-methyl}-4-phenylbutanoic acid (Example 44),

2-[(1-{[(5-methyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]-4-phenylbutanoic acid (Example 43),

cis-3-(2-methoxyethoxy)-2-[(1-{[(4-{[(phenylsulfonyl)amino]carbonyl}-cyclohexyl)amino]carbonyl}cyclopentyl)methyl]propanoic acid (Example 38),
(+)-2-{[1-({[2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-2-yl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 31),
(+)-2-[(1-{[(5-ethyl-1,3,4-thiadiazol-2-yl)amino]carbonyl}cyclopentyl)-methyl]pentanoic acid (Example 30),
2-({1-[(3-benzylanilino)carbonyl]cyclopentyl}methyl)pentanoic acid (Example 21),
2-[(1-{[(1-benzyl-6-oxo-1,6-dihydro-3-pyridinyl)amino]carbonyl}cyclopentyl)-methyl]-pentanoic acid (Example 22), and
2-{[1-({[(1R,3S,4R)-4-(aminocarbonyl)-3-butylcyclohexyl]amino}carbonyl)-cyclopentyl]methyl}pentanoic acid (Example 9).

29 Use of a compound as defined in any one of the preceding claims wherein the chiral carbon attached to R^1 is preferably the R-enantiomer.

30 Use of a NEPi compound according to any one of claims 1 to 29 for the treatment of MED wherein the medicament is administered systemically.

31 Use according to claim 30 wherein the medicament is administered orally.

32 Use of a pharmaceutical formulation including a NEPi compound together with a pharmaceutically acceptable excipient for the treatment of MED.

33. Use according to claim 32 wherein the NEPi compound is as defined in any of claims 2 to 29.

34. Use of a NEPi and a PDE5i for the treatment of MED.

35. Use according to claim 34 wherein the NEPi is as defined in any of claims 1 to 29 and wherein the PDE5i is selected from:

5-[2-ethoxy-5-(4-methyl-1-piperazinylsulphonyl)phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one (sildenafil) also known as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulphonyl]-4-methylpiperazine;

5-(2-ethoxy-5-morpholinoacetylphenyl)-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-n-propoxyphenyl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxyethoxy)pyridin-3-yl]-2-(pyridin-2-yl)methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

(+)-3-ethyl-5-[5-(4-ethylpiperazin-1-ylsulphonyl)-2-(2-methoxy-1(R)-methylethoxy)pyridin-3-yl]-2-methyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, also known as 3-ethyl-5-{5-[4-ethylpiperazin-1-ylsulphonyl]-2-([(1R)-2-methoxy-1-methylethyl]oxy)pyridin-3-yl}-2-methyl-2,6-dihydro-7H-pyrazolo[4,3-d] pyrimidin-7-one;

5-[2-ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-[2-methoxyethyl]-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one, also known as 1-{6-ethoxy-5-[3-ethyl-6,7-dihydro-2-(2-methoxyethyl)-7-oxo-2H-pyrazolo[4,3-d]pyrimidin-5-yl]-3-pyridylsulphonyl}-4-ethylpiperazine;

5-[2-*iso*-Butoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-(1-methylpiperidin-4-yl)-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

5-[2-Ethoxy-5-(4-ethylpiperazin-1-ylsulphonyl)pyridin-3-yl]-3-ethyl-2-phenyl-2,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one;

5-(5-Acetyl-2-propoxy-3-pyridinyl)-3-ethyl-2-(1-isopropyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

5-(5-Acetyl-2-butoxy-3-pyridinyl)-3-ethyl-2-(1-ethyl-3-azetidinyl)-2,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one;

(6R,12aR)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4-methylenedioxyphenyl) - pyrazino[2',1':6,1]pyrido[3,4-b]indole-1,4-dione (IC-351);

2-[2-ethoxy-5-(4-ethyl-piperazin-1-yl-1-sulphonyl)-phenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one (vardenafil) also known as 1-[[3-(3,4-dihydro-5-methyl-4-oxo-7-propylimidazo[5,1-f]-as-triazin-2-yl)-4-ethoxyphenyl]sulphonyl]-4-ethylpiperazine; and

the compound of example 11 of published international application WO93/07124 (EISAI).

36. Use according to claim 35 wherein the PDE5i is sildenafil.

37. A pharmaceutical composition comprising a NEPi and a PDE5i for the treatment of MED.

38. A kit comprising a first component and a second component for the treatment of MED wherein the first component comprises a NEPi and wherein the second component comprises a PDE5i.

39. The kit of claim 38 wherein the NEPi is as defined in any of claims 1 to 29.

40 Use of compounds which act via inhibiting the mechanism that terminates the biological activity of a number of bioactive peptides and in particular vasoactive peptides, more particularly neuropeptides, that are released during sexual arousal for the treatment of MED.

41 Use of compounds which acts via enhancing a non-NO dependant NANC pathway for the treatment of MED.

ABSTRACT

The present invention relates to the use of selective neural endopeptidase inhibitors for the treatment of male sexual dysfunction, in particular MED.

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